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APPLICATION

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TITLE: CENTROSOME PROTEINS AND USES THEREOF

APPLICANT: STEPHEN J. DOXSEY

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CENTROSOME PROTEINS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

This application claims benefit of priority from United States Provisional Patent Application Serial No. 60/410,520, filed on September 13, 2002, and to the United States Patent Application Serial No. (not yet assigned) filed on September 15, 2003, claiming priority therefrom, both of which patent applications are incorporated herein by reference in their entireties.

FIELD OF THE INVENTION

This invention relates to the centriolin and pericentrin-B/kendrin genes, the polypeptides they encode, and their uses in the detection, diagnosis, and treatment of centrosomal and cell division diseases and disorders.

BACKGROUND OF THE INVENTION

Centrosomes are the major microtubule nucleating organelles in most animal cells. They nucleate and organize microtubules for spindle assembly during mitosis and establish microtubule arrays in interphase cells for numerous cellular functions. Centrosomes are comprised of two major structural elements, centrioles and the pericentriolar material/centrosome matrix. Centrioles are microtubule barrels present as pairs in each centrosome, and appear to organize the pericentriolar material (Bobinnec *et al.*, *J. Cell Biol.*, 143:1575-1589, 1998) and anchor microtubules (Chretien *et al.*, *J Cell Biol.*, 120:117-133, 1997; Piel *et al.*, *J Cell Biol.*, 149:317-330, 2000). The pericentriolar material nucleates the growth of new microtubules and serves as a scaffold for molecules that regulate fundamental cellular processes.

SUMMARY OF THE INVENTION

The invention is based on the discovery that centriolin, together with pericentrin-B, plays a key roles in centrosome function. The invention provides new methods for diagnosing and treating centrosomal diseases.

The invention includes isolated nucleic acids that include SEQ ID NOs:1 and 3 (and complementary sequences, fragments, and analogs thereof), polypeptides encoded by SEQ ID

NOs:1 and 3 (and degenerate sequences, fragments, or analogs thereof), polypeptides that include SEQ ID NOs:2 and 4 (and fragments or analogs thereof (*e.g.*, those with conservative amino acid substitutions)), as well as biologically active fragments and analogs of any of SEQ ID NOs:1-4.

The invention also includes vectors comprising the nucleic acid molecules of SEQ ID NO:1 or 3, or fragments or analogs thereof), as well as cells comprising the nucleic acid molecules of SEQ ID NO:1 or 3, or fragments or analogs thereof.

The invention also features a method of reducing cell division by administering to a cell an amount of a centriolin or pericentrin-B modulator (*e.g.*, an RNAi, siRNA (e.g., any one of SEQ ID NOs:8-23), antisense nucleic acid, ribozyme, or antibody (including those produced either *in vivo* or *in vitro*, as well as monoclonal antibodies)) effective to disrupt microtubule organization in the cell, in which cell division is reduced. The invention can be used to treat cancer, leukemia, psoriasis, Hodgkin's disease, lymphoma, myelofibrosis, polycythemia vera, or other cell proliferative disorders or diseases.

Furthermore, the invention encompasses a method of treating abnormal centrosome function in a cell by administering to the cell an amount of centriolin polypeptide or pericentrin-B polypeptide effective to restore normal centrosome function.

A centriolin or pericentrin-B modulator is any molecule or other substance capable of increasing or decreasing centriolin or pericentrin-B expression, respectively. Such modulators include, for example, polypeptides, ribozymes, antisense molecules, siRNA molecules, antibodies, and small molecules.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The invention has a number of advantages. First, it provides the nucleic acid and polypeptide sequences of centriolin and pericentrin-B. These nucleic acid sequences themselves

have many uses, for example, in the production of hybridization probes to locate identical, homologous, or similar DNA or RNA sequences, within either humans or other organisms (especially mammals). The invention includes novel methods of modulating cell division, thus allowing treatment of associated diseases, disorders, or symptoms thereof, for which there have been few or no therapeutic options available. Another advantage provided by the invention involves methods for the diagnosis of abnormal centriolin and pericentrin-B expression or activity, thus allowing, for example, valuable insight into the etiology of diseases, disorders, or symptoms thereof that have previously defied medically useful explanation. Furthermore, the diagnosis of such diseases, disorders, or symptoms thereof can now be followed by previously unavailable methods of treatment. There are numerous advantages in addition to those listed here.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A is a representation of a series of SDS gels that show 35S(Methionine)-labeled HA-tagged centriolin (Cen) or empty vector (Vec) produced by in vitro translation and run on SDS gels directly or after immunoprecipitation with HA antibodies (Cen IP). Western blots were probed with anti-HA antibody showing overexpressed HA-tagged centriolin (HA-Cen) and its absence from cells overexpressing β-galactosidase (β gal). Centrosome fractions prepared from HeLa cells (panel 3) and *Xenopus* cells (XTC, panel 4) were blotted with antibodies to centriolin (Cen) and preimmune sera (PreI). Arrowheads show position of centriolin. In XTC cells, the band below centriolin appears to be a degradation product as a band of similar molecular weight is sometimes observed when the cDNA is translated in vitro or overexpressed. Bars represent positions of molecular weight markers ($x10^3$).

FIG. 1B depicts immunofluorescence images of endogenous centriolin in RPE1 cells through the cell cycle. Upper panels are merged images of centriolin (green), g tubulin (red) and nuclei (blue) from cells in G1, G2, proM, M and anaphase (A). Insets, higher magnifications of centrosomes stained for g tubulin (left panels) and centriolin (right panels). Staining of one of the two centrosomes is demonstrated most clearly in the G2 cell. Lower panels (Telo early, Telo

late) show separate images of g tubulin staining (left) and centriolin (right). In these images γ-tubulin marks the centrioles that have separated in some cases. Centriolin staining is confined to one of two centrioles in each centrosome and sometimes appears at the intercellular bridge (Telo early). Centrioles lacking centriolin are indicated by arrowheads in right panels. C, centriole, MB, midbody. Bar in bottom right of B, 10 mm for all except insets, 3 mm.

FIGs. 2A-G depict centriolin localized to maternal centrioles and noncentrosomal sites of microtubule anchoring.

FIG. 2A depicts HA-tagged centriolin overexpressed in COS-7 cells, which localizes to the centrosome (anti-HA, green) at the convergence of microtubules (red, anti-a tubulin). Bar, 10 μm.

FIG. 2B depicts an RPE1 cell immunostained with an antibody to polygluamylated tubulin (GT335) (Bobinnec *et al., J Cell Biol.,* 143:1575-1589, 1998) to label centrioles and the primary cilium (red) and for centriolin (green) that is localized to the maternal centriole (m) associated with the primary cilium (yellow in merge) but not on the daughter centriole (d). n, nucleus. Bar, 2 μm.

FIGs. 2C-E depict electron micrographs showing specific immunogold labeling of centriolin on subdistal appendages found on maternal but not daughter centrioles.

FIG. 2C depicts an electron micrograph showing a longitudinal section through maternal centrioles. An arrowhead shows striations characteristic of subdistal appendages. Bar, 100 nm.

FIG. 2D depicts an electron micrograph showing a cross-section through maternal centrioles. An arrowhead shows striations characteristic of subdistal appendages. Bar, 100 nm.

FIG. 2E depicts an electron micrograph showing a longitudinal section through both centrioles. The electron micrograph shows specific immunogold labeling of centriolin on subdistal appendages found on maternal (FIG. 2E, bottom) but not daughter centrioles (FIG. 2E, top). Bar, 100 nm.

FIG. 2F is a schematic diagram showing that centriolin is found at noncentrosomal sites of microtubule anchoring in pillar cells of the mouse cochlea (Mogensen *et al., Cell Motil Cytoskel,* 36:276-290,1997)(arrow)((F, G, arrows). T centrosome and associated cilium is shown schematically at top of cell.

FIG. 2G depicts a centriolin immunofluorescence staining overlaid with phase contrast image showing that centriolin is found at noncentrosomal sites of microtubule anchoring in pillar

cells of the mouse cochlea (Mogensen *et al., Cell Motil Cytoskel*, 36:276-290,1997)(arrow). Bar, 10 μm.

FIGs. 3A-G depict centriolin antibodies and protein overexpression, which disrupts cytokinesis.

FIG. 3A depicts a micrograph of *Xenopus* two cell embryos injected into one cell with ~50 nl anti-centriolin antibodies (2 mg/ml affinity purified) that fail to cleave (arrows) while noninjected cells (side opposite arrows) and cells injected with control rabbit IgG (2mg/ml, top) cleave normally.

FIG. 3B is a graph depicting the quantification of results from injection experiments (average of 3 individual experiments). Unless otherwise specified for these and other experiments, n is the total number of cells counted.

FIG. 3C is a micrograph of a COS-7 cell in cytokinesis overexpressing HA-tagged centriolin (anti-HA, inset) showing persistent microtubule bundles in the intercellular bridge (main panel, α-tubulin staining) despite reformation of the nucleus and decondensation of chromatin (DNA, inset). Bar, 15 μm, and for insets, 35 μm.

FIG. 3D is a micrograph of a control COS-7 cell expressing HA alone (left inset) shows a narrow intercellular bridge and greatly diminished microtubule polymer (main panel), characteristic of cells that have reformed nuclei and decondensed DNA (right panel). Bar, 15 μm, and for insets, 35 μm.

FIG. 3E is a graph depicting a quantitative analysis showing the proportion of telophase cells in the presence of HA-centriolin of HA alone. Results are the average of 2 independent experiments. Unless otherwise specified for these and other experiments, n is the total number of cells counted.

FIG. 3F is a graph depicting a quantitative analysis showing the proportion of aberrant telophase cells (F, persistent microtubule bundles in late telophase/early G1) in the presence of HA-centriolin of HA alone. Results are the average of 2 independent experiments. Unless otherwise specified for these and other experiments, n is the total number of cells counted.

FIG. 3G is a graph depicting a quantitative analysis showing the proportion of binucleate cells in the presence of HA-centriolin of HA alone. Results are the average of 2 independent experiments. Unless otherwise specified for these and other experiments, n is the total number of cells counted.

FIGs. 4A-G depict siRNAs targeting centriolin, which induces cytokinesis defects and delays in RPE cells.

FIG. 4A depicts an RT-PCR analysis showing that centriolin mRNA is reduced in RPE1 cells treated with centriolin-specific siRNAs but is unaffected in cells treated with siRNAs targeting lamins A/C (left, sequence identity confirmed). Control (a tubulin) RT-PCR was performed in the same reaction mixtures with centriolin and lamin (bottom panels).

FIG. 4B depicts immunofluorescence images of cells treated with siRNAs targeting centriolin showing a cell with reduced centriolin at the centrosome/centriole (green/white, lower) and a cell that is unaffected by the treatment (upper). Centrosomes are co-labeled with γ-tubulin (red). Insets: higher magnification of centrosomes in upper and lower cells. Bar, 12 μm, and for insets, 2 μm.

FIG. 4C depicts a graph showing the proportion of cells whose centrosomal centriolin staining is undetectable or markedly reduced in cells treated with centriolin and GFP siRNAs.

FIG. 4D depicts average fluorescence intensities at individual centrosomes in cells treated with lamin or centriolin siRNAs. The centrosome fluorescence in most centriolin siRNA-treated cells (83%, left of arrow) was below the lowest values observed in control cells.

FIG. 4E is a graph showing the percentage of abnormal telophase (wide intercellular bridges, cells that remain interconnected in G1) following treatment with centriolin siRNAs versus lamin siRNAs.

FIG. 4F is a graph showing the percentage of binucleate cells following treatment with centriolin siRNAs versus lamin siRNAs.

FIG. 4G is a series of graphs showing that progression of cells through prom/M in cells treated with centriolin siRNAs is similar to those treated with lamin siRNAs, while progression through telophase is delayed.

FIGs. 5A-E depict centriolin domain structure and interaction with yeast Bub2p.

FIG. 5A is a schematic diagram showing centriolin coiled coil regions (boxes), noncoiled regions (lines) and domains homologous to budding and fission yeast Nud1p and Cdc11p, human stathmin and human and Drosophila TACC proteins.

FIG. 5B depicts an alignment of the centriolin Nud1 domain (SEQ ID NO:7) with the yeast Nud1p (SEQ ID NO:6)/Cdc11p (SEQ ID NO:5) proteins.

FIG. 5C depicts the position of the Nud1 domain and Cdc11p in centriolin and the homologous domains within the yeast proteins.

FIG. 5D is a depiction showing that the Nud1 domain interacts with budding yeast Bub2p by yeast two-hybrid analysis. The blue colony in blue box (middle bottom) and increased β-galactosidase activity (Bar 2 of graph) demonstrate a specific interaction between the human centriolin Nud1 domain (hNud1) and Bub2p. Bar 1, LexA- BUB2 x transactivation domain (TAD), Bar 2, LexA-BUB x TAD-hNud1, Bar 3, LexA-BFA1 x TAD, Bar 4, TAD-hNud1 x LexA-BFA1, Bar 5, TAD-hNud1 x LexA.

FIG. 5E depicts specific co-immunoprecipitation of HA-tagged hNud1 and Lex A-tagged yeast Bub2p from budding yeast cells. Co-precipitation was observed using antibodies to either protein and only when both were co-expressed (top middle panel in each group).

FIGs. 6A-E depict overexpression of the centriolin Nud1 domain, which induces cytokinesis defects but does not mislocalize centrosomal centriolin.

FIG. 6A depicts a micrograph showing the Nud1-GFP domain of centriolin expressed in a COS-7 cell. Bar, 10 μm.

FIG. 6B depicts a micrograph showing that the Nud1-GFP domain of centriolin expressed in a COS-7 cell does not affect localization of endogenous centriolin to centrosomes. Bar, 10 μm.

FIG. 6C depicts an image showing two nuclei (blue) in a cell expressing the Nud1-GFP domain of centriolin (green, appears yellow in merge) colabeled with microtubules (red). Bar, 15 μm.

FIG. 6D is a graphs showing percent telophase in cells overexpressing Nud1-GFP domain compared with nontransfected cells.

FIG. 6D is a graphs showing percent binucleates in cells overexpressing Nud1-GFP domain compared with GFP-expressing cells.

FIGs. 7A-G depict pericentrin-B, which co-localizes with centriolin, and reduction in its levels, which mislocalizes centriolin and induces delays and defects in cytokinesis.

FIG. 7A depicts an RPE cell showing pericentrin-B (red) colocalizing with centriolin (green, yellow in merge) at centrosomes (arrow) and intercellular bridges in telophase (second panel). Bar, 15 μm.

FIG. 7B depicts an RT-PCR analysis showing that pericentrin-B (Pcnt-B) siRNA treatment reduces pericentrin-B mRNA levels (upper left) while lamin siRNA has no effect (upper right, sequence confirmed). A tubulin RT-PCR was performed in the same reaction mixtures with pericentrin-B and lamin and serves as a control (bottom panels). Pcnt-B, pericentrin-B.

FIG. 7C is a graph showing the proportion of cells whose centrosomal staining for pericentrin-B is undetectable or markedly reduced in cells treated with pericentrin-B and GFP siRNAs. Pcnt-B, pericentrin-B.

FIG. 7D is a graph showing pericentrin-B fluorescence intensity at individual centrosomes (thin bars) in cells treated with lamin or pericentrin-B siRNAs. The centrosome fluorescence in most pericentrin-B siRNA-treated cells (87%, left of arrow) was below the lowest levels detected in control cells (lamin). Pcnt-B, pericentrin-B.

FIG. 7E depicts a micrograph of immunofluorescence images showing that pericentrin-B siRNA-treated cells with reduced centrosomal pericentrin-B (red, arrow) also have reduced levels of centriolin (yellow, arrow). Unaffected cell (bottom) stains for both proteins. Bar, 15 μm.

FIG. 7F is a graph showing the percentages of HeLa cells in telophase following treatment with pericentrin-B siRNAs and GFP siRNAs. Bars in A, E 15 mm. Pcnt-B, pericentrin-B.

FIG. 7G is a graph showing the percentages of aberrant telophase cells following treatment with pericentrin-B siRNAs and GFP siRNAs. Pcnt-B, pericentrin-B.

FIGs. 8A-G depict siRNAs targeting centriolin and pericentrin-B, which induces G1/G0 arrest.

FIG. 8A is a graph showing that RPE1 cells treated with centriolin or pericentrin-B siRNAs (blue line) do not shift into the G2/M peak following nocodazole treatment as seen for control lamin siRNA-treated cells (red line).

FIG. 8B is a graph showing that RPE1 cells treated with centriolin or pericentrin-B siRNAs (blue line) do not shift into the G2/M peak following nocodazole treatment as seen for control lamin siRNA-treated cells (red line). Pcnt-B, pericentrin-B.

FIG. 8C is a graph showing that the centriolin and pericentrin-B siRNA phenotype is similar to that observed in serum-starved cells (blue, serum starved; red, not starved).

FIG. 8D depicts a micrograph of Ki-67 staining of pericentrin-B siRNA-treated cells showing that a cell lacking pericentrin-B also lacks Ki-67 (arrowhead), compared with a cell that is unaffected (upper left) and stains for both pericentrin-B (red, arrow) and Ki-67 (green). Bar, 7.5 μm.

FIG. 8E is a graph showing quantification of Ki-67 staining in cells treated with siRNAs targeting pericentrin-B, centriolin and GFP. Pcnt-B, pericentrin-B.

FIG. 8F is a graph showing that HeLa cells, with abrogated p53 function, did not arrest when centriolin or pericentrin-B levels were reduced. The majority stained for Ki-67 and shifted into the G2 peak when treated with nocodazole.

FIG. 8G is a graph showing that Saos-2 cells, with abrogated p53 function, did not arrest when centriolin or pericentrin-B levels were reduced. The majority stained for Ki-67 and shifted into the G2 peak when treated with nocodazole.

FIG. 9 is a table that shows cell cycle analysis of flow cytometry data without nocodazole (above) and with nocodazole (below) (see FIGs. 8A-G).

FIG. 10 depicts micrographs that show that lamin siRNA does not affect pericentrin-B localization at centrosomes. Images showing that cells in which lamin is disrupted by lamin-specific siRNAs (upper left) have normal levels of pericentrin-B (lower left), similar to cells unaffected by lamin-specific siRNAs (right, both panels). Insets, higher magnifications of centrosomes in lower panel. Bar, 7.5 μm, and Bar, 2 μm for insets. Pcnt-B, pericentrin-B.

FIG. 11 is a graph that that shows that reduction in pericentrin-B levels specifically mislocalizes centrosomal centriolin. Quantification of cells whose centrosomal levels of the stated antigens are not detectably altered following treatment with siRNAs targeting pericentrin-B (Pcnt-B). n = total number of cells.

FIGs. 12A-I depict the nucleotide sequence (SEQ ID NO:1) (above) and amino acid sequence (SEQ ID NO:2) (below) of centriolin.

FIG. 13A-H depict the nucleotide sequence (SEQ ID NO:3) (above) and amino acid sequence (SEQ ID NO:4) (below) of pericentrin-B.

FIG. 14 is a schematic diagram of centriolin. It depicts centriolin domains that share homology with budding and fission yeast proteins Nud1p and Cdc11p, human stathmin, and human and Drosophila TACCs (above). Percentages represent identities and similarities of

centriolin to the homologous proteins described above. It also depicts centriolin regions predicted to be coiled coil (below). Beneath each sequence are centriolin amino acid numbers.

FIGs. 15A-N are graphs and micrographs relating to RPE-1 cells treated with siRNAs targeting centriolin.

FIG. 15A is a graph showing the dramatic increase in the percentage of cells in telophase/cytokinesis after siRNA targeting of centriolin (~70-fold).

n = total number of cells counted (FIG. 15A-D).

FIG. 15B is a graph showing the increased percentage of binucleate cells in a HeLa cell line after treatment with centriolin siRNAs (4-15-fold greater than controls). Values represent data from a single experiment. The time analyzed was as follows: 48 hours and 72 hours.

FIG. 15C is a graph showing the increased percentage of binucleate cells in a U2OS cell line after treatment with centriolin siRNAs (4-15-fold greater than controls). Values represent data from a single experiment. The time analyzed was as follows: U2OS, 72 hours.

FIG. 15D is a graph showing the increased percentage of binucleate cells in an RPE-1 cell line after treatment with centriolin siRNAs (4-15-fold greater than controls). Values represent data from a single experiment. The time analyzed was as follows: RPE-1, 24 hours.

FIGs. 15E-J depict micrographs showing that microtubule organization (FIGs. 15E-J) and microtubule nucleation (FIGs. 15K and L) are not detectably altered in cells treated with centriolin siRNA (FIGs. 15F, H, J, and L) compared with cells treated with lamin siRNA (FIGs. 15E, G, I, and K). No centrosome staining is observed in centriolin siRNA-treated cells.

FIG. 15E depicts a micrograph showing microtubule organization of cells (in interphase) treated with lamin siRNA. Arrow indicates position of centrosome. Microtubules (red), centrosomes (green/yellow), and nuclei (blue). MB = midbody. Bar in FIG. 15N, 5 μm.

FIG. 15F depicts a micrograph showing microtubule organization of cells (in interphase, lower cell) treated with centriolin siRNA. Microtubules (red), centrosomes (green/yellow), and nuclei (blue). MB = midbody. Bar in FIG. 15N, 5 μm.

FIG. 15G depicts a micrograph showing microtubule organization of cells (prometaphase/metaphase (proM/M)) treated with lamin siRNA. Arrows indicate positions of centrosomes. Microtubules (red), centrosomes (green/yellow), and nuclei (blue). MB = midbody. Bar in FIG. 15N, 5 μm.

FIG. 15H depicts a micrograph showing microtubule organization of cells (prometaphase/metaphase (proM/M)) treated with centriolin siRNA. Microtubules (red), centrosomes (green/yellow), and nuclei (blue). MB = midbody. Bar in FIG. 15N, 5 μm.

FIG. 15I depicts a micrograph showing microtubule organization of cells (telophase) treated with lamin siRNA. Arrows indicate positions of centrosomes. Microtubules (red), centrosomes (green/yellow), and nuclei (blue). MB = midbody. Bar in FIG. 15N, 5 μm.

FIG. 15J depicts a micrograph showing microtubule organization of cells (telophase) treated with centriolin siRNA. Microtubules (red), centrosomes (green/yellow), and nuclei (blue). MB = midbody. Bar in FIG. 15N, 5 μm.

FIG. 15K depicts a micrograph showing microtubule nucleation of cells treated with lamin siRNA. Arrow indicates position of centrosome. Insets is an enlargement of centriolin staining at centrosomes (or microtubule convergence sites) at arrows. Bar in FIG. 15N, 5 μm (including inset).

FIG. 15L depicts a micrograph showing microtubule nucleation of cells treated with centriolin siRNA. Arrow indicates position of centrosome. Insets is an enlargement of centriolin staining at centrosomes (or microtubule convergence sites) at arrows. Bar in FIG. 15N, 5 μm (including inset)

FIG. 15M and N depicts cells treated with siRNAs targeting centriolin stained for microtubules (red) and DNA (blue). Arrowheads indicate contiguous connections between two or more cells.

FIG. 15M depicts three interconnected cells forming a syncytium. Cells were treated with siRNAs targeting centriolin stained for microtubules (red) and DNA (blue). Arrowheads indicate contiguous connections between two or more cells. Bar, 15 μm.

FIG. 15N depicts one daughter of an interconnected pair of cells that has reentered mitosis (M). Cells were treated with siRNAs targeting centriolin stained for microtubules (red) and DNA (blue). Arrowhead indicates contiguous connections between two or more cells. Bar, 15 μm.

FIGs. 16A-F are depictions of time-lapse images of HeLa cells treated with centriolin siRNAs, revealing unique cytokinesis defects. Time in hours and minutes is included in each panel in FIGs. 16A-D. Time-lapse videos (Videos 1-3) of the series of images shown in FIGs. 16A-C are available at http://www.jcb.org/cgi/content/full/jcb.200301105/DC1.

FIG. 16A depicts a cell treated with control siRNAs targeting lamin moving apart, forming visible midbodies (arrow), and completing the final cleavage event with normal timing (1-3 hours after metaphase). Bar, 10 μm.

FIGs. 16B-D depict centriolin siRNA-treated cells that remain attached for extended periods of time through persistent intercellular bridges and sometimes do not show visible midbodies.

FIG. 16B depicts a cell that remains attached by a long intercellular bridge for at least 8 hours. The cell cleaves, both daughter cells round up, and at least one appears to undergo apoptotic cell death (upper cell, 7:20, extensive blebbing and decrease in size). Bar, 10 μm.

FIG. 16C depicts a dividing cell that has not completed cleavage and reenters the next mitosis. One cell rounds up and is drawn to the other. The other rounds-up and both undergo the early stages of cytokinesis to form a total of four cells; these progeny often remain attached by intercellular bridges forming syncytia. Bar, 10 μm.

FIG. 16D depicts a cell showing three failed attempts at cell cleavage over a 9.5-hour time period. Bar, 10 μm.

FIG. 16E is a graph showing time quantitation of cells treated with siRNAs targeting centriolin progress from nuclear envelope breakdown (NEB) to anaphase with normal timing, similar to lamin siRNA controls. Vertical bars represent recordings from single cells. Results represent recordings of individual cells from several independent experiments.

FIG. 16F is a graph showing time quantitation of centriolin siRNA-treated cells that are delayed in cytokinesis (~70%) compared with control lamin siRNA-treated cells, a value consistent with a 70-80% silencing efficiency. Vertical bars represent recordings from single cells. Results represent recordings of individual cells from several independent experiments.

FIG. 17 is a depiction of immunofluorescence images showing two microtubule asters near a single nucleus (N) in a cell from an embryo injected with control IgG (panel above), and two nuclei and two asters in a cell from a centriolin antibody-injected cell (panel below, microtubules stained with anti-α tubulin). Bar, 10 μm.

Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION OF THE INVENTION

The invention is based, in part, on the discovery of the interaction between centrosome polypeptide components, centriolin and pericentrin-B, whose disruption induces cytokinesis delays/defects and subsequent G1/G0 arrest. This phenotype is essentially the same as that observed when centrosomes are experimentally eliminated from vertebrate cells (Hinchcliffe *et al., Science,* 291:1547-1550, 2001; Khodjakov and Rieder, *J Cell Biol.,* 153:237-242, 2001). Taken together, these results demonstrate that centriolin and pericentrin-B represent core centrosome components required for progression through cytokinesis and entry into S phase.

Yeast Centrosomes

To ensure that each daughter cell receives one copy of each chromosome during cell division, exit from mitosis and cytokinesis must occur only after chromosomes are properly segregated. It is therefore essential that these events are precisely ordered. A role for centrosomes in defining the site of cell cleavage during cytokinesis has been suggested for some time (Rappaport, *Int Rev Cyctol,*105:245-281, 1986). Recent studies with vertebrate cells provide evidence for a direct link between centrosome activity and completion of cytokinesis. Elimination of centrosomes from interphase cells by removal with a microneedle (Hinchcliffe *et al., Science,* 291:1547-1550, 2001) or from mitotic cells by laser ablation (Khodjakov and Rieder, *J Cell Biol.,* 153:237-242, 2001), caused cytokinesis defects, arrest, or failure. It was also shown that during the final stages of cytokinesis the maternal centriole moved to the intercellular bridge, the microtubule-filled interconnection between nascent daughter cells. Centriole re-positioning correlated with bridge narrowing and microtubule depolymerization, and its subsequent movement away from the bridge correlated with cell cleavage. Taken together, these studies suggest that centrosomes either activate the final stages of cytokinesis or induce release of cells from a checkpoint that monitors completion of mitosis.

These studies are consistent with work in both fission and budding yeast that show that spindle pole body-anchored signaling pathways regulate cytokinesis. In budding yeast, a regulatory pathway called the mitotic exit network (MEN) controls anaphase progression by inactivating cyclin-dependent kinases (CDKs, for reviews see Bardin and Amon, *Nat Rev Mol Cell Biol,* 2:815-826, 2001; McCollum and Gould, *Trends Cell Biol.,* 11:89-95, 2001; Pereira and Schiebel, *Curr Opin Cell Biol.,* 13:762-769, 2001). Cells mutant for this GTPase-driven

signaling network typically arrest in late anaphase. As cells enter late anaphase the spindle pole body (centrosome equivalent) moves into the nascent daughter cell (bud). This event brings into contact a GTP binding protein called Tem1p located on the spindle pole body nearest the bud and the guanine nucleotide exchange factor Lte1p located in the bud. Tem1p is converted to its active GTP form by Lte1p probably in combination with inactivation of the Bub2p GTPase activating protein (GAP). Activation of Tem1p, together with loss of microtubule contact with the bud neck and microtubule depolymerization at the bud neck, triggers exit from mitosis (Bardin *et al., Cell,* 102:21-31, 2000; Bloecher *et al., Nat Cell Biol.,* 2:556-558, 2000; Gruneberg *et al., Embo J,* 19:6475-6488, 2000; Adames *et al., J Cell Biol.,* 153:159-168, 2001). In addition to regulating mitotic exit, the MEN also appears to play a role in cytokinesis (see Bardin and Amon, *Nat Rev Mol Cell Biol,* 2:815-826, 2001; Guertin *et al., Microbiol Mol Biol Rev.,* 66:155-178, 2002; McCollum and Gould, *Trends Cell Biol.,* 11:89-95, 2001).

In fission yeast, a signal transduction pathway analogous to the MEN has been termed the septation initiation network (SIN) (see Bardin and Amon, *Nat Rev Mol Cell Biol,* 2:815-826, 2001; Le Goff *et al., Curr Genet,* 35:571-584, 1999a; McCollum and Gould, *Trends Cell Biol.,* 11:89-95, 2001). This pathway is anchored at the spindle pole body by Cdc11p, a protein homologous to the budding yeast Nud1p, and by sid4p (Chang and Gould, *Proc Natl Acad Sci U S A,* 97:5249-5254, 2000; Krapp *et al., Curr Biol.,* 11:1559-1568, 2001; Tomlin *et al., Mol Biol Cell,* 13:1203-1214, 2002). SIN mutants fail in cytokinesis but continue to replicate their DNA and become elongated and multinucleate (Balasubramanian *et al., Genetics,* 149:1265-1275, 1998; Fankhauser *et al., Cell,* 82:435-444, 1995). A SIN-dependent checkpoint was recently identified that monitors completion of cytokinesis (Le Goff *et al., Mol Gen Genet.,* 262:163-172, 1999b; Liu *et al., J Cell Sci,* 113:1223-1230, 2000; Trautmann *et al., Curr Biol.,* 11:931-940, 2001). Cytokinesis failure causes cells to arrest prior to the next mitosis with two nuclei, thus preventing propagation of polyploid cells. Like the MEN, the SIN also may play a role in mitotic exit through inactivation of mitotic CDKs under certain circumstances (see Bardin and Amon, *Nat Rev Mol Cell Biol,* 2:815-826, 2001; McCollum and Gould, *Trends Cell Biol.,* 11, 89-95, 2001). Thus, both the SIN and MEN appear to regulate two steps in the latter stages of mitosis, mitotic exit and completion of cytokinesis. Consistent with their shared roles in mitosis, several components of the SIN and MEN share sequence homology (see Guertin *et al., Microbiol Mol Biol.,* 66:155-178, 2002).

Vertebrate Centrosomes

Although some of the late events in vertebrate cell mitosis appear similar to those in yeasts, there is little evidence for the existence of a signaling pathway homologous to the MEN or SIN in vertebrate cells. Although a few components related to yeast proteins have been identified in vertebrates, they have not been shown to possess activities and interactions of the SIN and MEN pathways (Cuif *et al., Embo J,* 18:1772-1782, 1999; Hirota *et al., J Cell Biol.,* 149:1073-1086, 2000; Luca and Winey, *Mol Biol Cell,* 9:29-46, 1998; Mailand *et al., Nat Cell Biol.,* 4:318-322, 2002).

In addition to their role in cytokinesis, centrosomes play a role in cell cycle progression. When centrosomes were removed from vertebrate cells, half completed mitosis but did not initiate DNA replication (Hinchcliffe *et al., Science,* 291:1547-1550, 2001; Khodjakov and Rieder, *J Cell Biol.,* 153:237-242, 2001). The authors suggested that centrosomes controlled entry into S phase by recruiting or concentrating "core" centrosome molecules required for this process or that they indirectly activated a cellular checkpoint that monitored aberrant centrosome number. Consistent with the checkpoint model are results from fission yeast showing that cytokinesis failure triggers a checkpoint that inhibits entry into the following mitotic cycle (see above). Moreover, vertebrate cells treated with cytochalasin D to inhibit actin-mediated cell cleavage, become tetraploid and subsequently arrest in G1 (Andreassen *et al., Mol Biol Cell,* 12:1315-1328, 2001). Elucidation of the mechanism by which centrosomes contribute to cell cycle progression in vertebrate cells will require identification of the molecular components and pathways that control these events.

The current invention is based, in part, on the identification of a novel human core centrosome/centriole component called centriolin. Modification of centriolin levels produces precisely the same phenotype observed when centrosomes are experimentally eliminated from cells, namely cytokinesis defects and G1 arrest. Centriolin shares homology with the yeast MEN/SIN components Nud1p/cdc11, is able to bind a yeast MEN component that interacts with Nud1p (Bub2), and is anchored to centrosomes by pericentrin-B, analogous to the anchoring of the SIN component cdc11 to spindle pole bodies by sid4. Reduction in levels of centriolin or pericentrin-B induces G1/G0 arrest.

The invention is based, at least in part, on the interaction of centriolin with pericentrin-B as components of a regulatory pathway in vertebrate cells that control progression through cytokinesis and into S phase. This invention discloses a molecular mechanism for cytokinesis defects and G1 arrest in vertebrate cells whose centrosomes are experimentally eliminated. It identifies core centrosome proteins involved in the final stages of cytokinesis that are members of a regulatory pathway analogous to the yeast SIN and MEN. It also demonstrates that reduction in expression or activity of these proteins arrests cells in G1/G0.

Identification of a Vertebrate Pathway that Regulates Cytokinesis

Regulatory pathways that control mitotic exit and cytokinesis have been described in budding and fission yeasts, but an analogous pathway in vertebrate cells has not been previously identified. The present invention elucidates such a pathway. Alteration of centriolin or pericentrin-B levels in vertebrate cells affects progression through cytokinesis just as mutation or overexpression of SIN or MEN proteins affects mitotic exit and cytokinesis in budding and fission yeasts. Centriolin shares homology with spindle pole body proteins in budding and fission yeasts (Nud1p, Cdc11p) that anchor downstream effectors of the MEN and SIN. Like Nud1p, the centriolin Nud1-related domain interacts with the MEN GAP, Bub2p. Both yeast and human proteins localize to microtubule organizing centers (centrosomes, spindle pole bodies). Overexpression of the Nud1-related domain of centriolin disrupts cytokinesis suggesting that it sequesters vertebrate homologue(s) of the MEN/SIN. Centriolin localizes to the maternal centriole and is translocated to the intercellular bridge during cytokinesis. It has recently been postulated that translocation of the maternal centriole to and subsequently away from the intercellular bridge is required to activate a regulatory network that controls cytokinesis in vertebrates in much the same way that movement of the spindle pole body into the bud triggers the MEN in budding yeast (Piel *et al., Science,* 291:1550-1553, 2001). This places centriolin at the right place at the right time to affect progression through the late stages of cytokinesis. Pericentrin-B anchors centriolin to centrosomes and reduction in its level mislocalizes centrosomal centriolin resulting in cytokinesis defects, just as loss or mutation of the fission yeast Sid4p mislocalizes Cdc11p and perturbs cytokinesis. Taken together, these results suggest a model in which centriolin and pericentrin-B define a vertebrate pathway analogous to the yeast

MEN and SIN that is required for completion of cytokinesis. This pathway will be referred to as the cytokinesis activation network (CAN).

The vertebrate CAN appears to be more like the SIN than the MEN. Cells with defects in the SIN fail to septate but exit mitosis and grow to form elongated multinucleate cells. Similarly, vertebrate cells defective for the CAN often fail to cleave forming large binucleate cells. Like fission yeast, vertebrate cells with cytokinesis defects retain microtubule structures and midbodies suggesting that the defect is at the final stages of cell cleavage. In contrast, budding yeast cells defective for MEN function arrest in anaphase.

The terminal stage of arrest for these three pathways is different. Budding yeast arrest in late anaphase while vertebrate cells arrest in the following G1/G0. Although fission yeast do not undergo cell cycle arrest, recent studies have uncovered a SIN-dependent cytokinesis checkpoint that arrests cells in G2 when they fail to undergo septation and cytokinesis (see Bardin and Amon, *Nat Rev Mol Cell Biol.*, 2:815-826, 2001; McCollum and Gould, *Trends Cell Biol.*, 11:89-95, 2001). It is interesting to compare how similar pathways control mitotic exit, cytokinesis, and cell cycle progression in these different organisms.

Mechanism of Centriolin-Induced Cytokinesis Defects

Centriolin function was targeted using multiple strategies, cell types, and species. All produced cytokinesis defects including reduction and elevation of centriolin levels, ectopic expression of the centriolin Nud1-related domain, ectopic expression of the pericentrin-B centrosome targeting domain, injection of centriolin antibodies, and reduction of pericentrin-B levels. Although the precise cause for an effect on cytokinesis is not yet known, one possibility is that the effect is achieved by modulating localization of centriolin-bound components of the CAN. Perhaps the most compelling data supporting this model is that overexpression of the centriolin Nud1-related domain induces cytokinesis delays and defects. This domain binds to yeast Bub2p, lacks the potential microtubule modifying domains (stathmin, TACCs), and does not disrupt centrosomal localization of endogenous centriolin when overexpressed. Overexpression of this domain in vertebrate cells may sequester a vertebrate homolog of the MEN/SIN and affect progression of cytokinesis.

Centrosome Integrity Checkpoint

The invention is based, in part, on the discovery that vertebrate cells with reduced centriolin or pericentrin-B levels exhibit cytokinesis defects and subsequently arrest in G1/G0. Cytokinesis defects and G1 arrest occur when centrosomes are experimentally eliminated from vertebrate cells. One of the discoveries upon which this invention is based is of a different mechanism for centrosome-mediated G1 arrest: activation of a checkpoint that monitors centrosome composition and/or function. Several observations support this. Reduction in levels of centrosome proteins that have no detectable effect on cytokinesis cause a potent G1/G0 arrest, indicating that the arrest is unrelated to cytokinesis. Several centrosome proteins that function in pathways unrelated to the CAN/MEN/SIN induce G1/G0 arrest, demonstrating that the arrest is unrelated to this regulatory pathway. Even noncentrosome proteins that affect centrosome assembly and integrity induce G1/G0 arrest, such as members of the dynactin and dynein complexes.

G1 arrest is specific for centrosomes, as shown by the fact that targeting of proteins of other cellular organelles, such as the nucleus (*e.g.*, lamin A/C), has no detectable effect on cell cycle progression. Alterations in centrosome composition, integrity, or function induce G1/G0 arrest even in cells with normal centrosome numbers. This demonstrates that vertebrate cells have a centrosome integrity checkpoint that monitors the composition and/or function of centrosomes. Inactivation of this pathway can lead to cytokinesis failure, aneuploidy, and tumor progression.

Centrosome Defects and the Induction of Exit from the Cell Cycle (G0)

Cells lacking centrosomes are unable to enter S phase and are arrested in G1 or G1/S (the restriction point). However, the invention is based, at least in part, on the discovery that cells with reduced centrosome protein levels exit the cell cycle and enter a quiescent G0 state. These cells were negative for antibodies that recognize cycling or cell cycle arrested cells, and not G0 or differentiated cells. Flow cytometry patterns in these cells were indistinguishable from serum-starved cells but different from cells in G1 or those arrested in G1/S. The G0 arrest phenotype was observed in multiple cell types including normal diploid cell lines (*e.g.*, RPE, BJ-1), two genetically unstable tumor or tumor-like cell lines (*i.e.*, HeLa, COS), cell lines with normal p53 (*i.e.*, RPE, BJ-1) and altered p53 (*i.e.*, HeLa), and cells of different tissue origins and species

(*e.g.*, human, monkey, mouse, hamster). Because the G0 state is more permanent and less subject to being overridden or reversed than cells arrested within the cell cycle, it can be exploited in cancer therapeutics by targeting centrosome proteins in tumor cells to induce exit from the cell cycle, and perhaps trigger re-differentiation.

Mechanism of G1/G0 Arrest

G1 arrest occurs when entire centrosomes are physically eliminated from vertebrate cells by laser ablation or microsurgery. It was demonstrated that even minor changes in centrosome composition can effectively inhibit cell cycle progression. However, the mechanism by which cells arrest occurs has been unknown. Possibilities include that the arrest is a downstream consequence of improper exit from cytokinesis/mitosis, that the CAN may control entry into S phase in addition to its role in cytokinesis, or that centriolin and pericentrin-B have dual roles in cytokinesis and S phase entry. The results of experiments carried out as part of the invention suggest that loss of centrosomal centriolin or pericentrin-B activates a checkpoint that monitors centrosome integrity (or number), thus preventing cells from entering S phase.

The G1/G0 arrest induced by reduction of centriolin or pericentrin-B is p53-dependent. The p53 pathway is also activated when cytokinesis is prevented by inhibiting actin ring formation or by overexpression of aurora-A kinase. The mechanism of p53 activation under these different experimental conditions is not yet understood.

Additional Roles for Centriolin and Pericentrin-B

Centriolin and pericentrin-B perform functions in addition to their roles in regulating completion of cytokinesis. Both are extraordinarily large proteins (260 kD, 350 kD, respectively) with a diversity of functional domains. Like other large centrosome-coiled coil proteins, both act as scaffolds for anchoring numerous proteins and activities. Centriolin has domains that may affect microtubule stability (stathmin, TACC) and microtubule anchoring (Nud1p). The microtubule bundles and other changes in microtubule organization observed in cells overexpressing full-length centriolin result from activation or suppression of these microtubule-modifying domains, and these microtubule structures are not observed when the Nud1-related domain is overexpressed. Additional functions for centriolin and pericentrin-B are also suggested by their re-localization from centrosomes to the intercellular bridge and midbody

during cytokinesis, as this redistribution does not occur in the putative yeast homologues (Chang and Gould, *Proc Natl Acad Sci U S A,* 97:5249-5254, 2000; Gruneberg *et al., Embo J,* 19:6475-6488, 2000; Krapp *et al., Curr Biol.,* 11:1559-1568, 2001).

Pericentrin-B shares homology with budding yeast spc110p, a spindle pole body protein that anchors g tubulin complexes (Flory *et al., Proc Natl Acad Sci U S A,* 411:494-498, 2000), and appears to be an isoform of pericentrin-A. Pericentrin-B may interact with the same molecules as pericentrin-A including g tubulin complexes (Dictenberg *et al., J Cell Biol.,* 141:163-174, 1998), PKA (Diviani *et al., Curr Biol.,* 10:417-420, 2000), and cytoplasmic dynein (Purohit *et al., J Cell Biol.,* 147:481-491, 1999). However, reduction of pericentrin-B levels does not prevent centrosomal association of g tubulin as would be expected if it interacted with the g tubulin complex.

Centriolin is a Marker for Maternal Centrioles and Centriole Maturation

Even though centrioles begin duplicating at G1/S, centriolin does not significantly accumulate at the new mother centriole until mitosis. It thus serves as a marker for centriole maturation, a characteristic shared with cenexin (Lange and Gull, *J Cell Biol.,* 130:919-927, 1995) and ninein (Mogensen *et al., J Cell Sci,* 113:3013-3023, 2000). Localization of centriolin to the subdistal appendages suggests that it may be involved in recruitment of other CAN components to these sites. It may also function in the anchoring of microtubules at these sites (Piel *et al., J Cell Biol.,* 149:317-330, 2000), or in maintaining the integrity of the appendages. Localization of centriolin to appendages could also facilitate its movement from the centriole to the intercellular bridge via microtubules anchored at these sites.

Genetic Instability Induced by Reduction in Centriolin or Pericentrin-B Levels

The formation of binucleate cells in cells with reduced centriolin or pericentrin-B demonstrates that these proteins affect the fidelity of partitioning chromosomes into daughter cells by interfering with cytokinesis. Because centrosome anomalies are implicated in the establishment of genetic instability associated with human tumors, functional changes in centriolin or pericentrin-B that affect centrosomes contribute to genetic instability. Moreover, the location of the centriolin gene to a chromosomal site often involved in translocations, and its

presence as an oncogenic fusion protein with FGF-R, suggests another mechanism whereby centriolin can influence tumor progression.

Nucleic Acids

The invention encompasses nucleic acids that have sequences substantially identical to any one of the nucleic acid sequences of SEQ ID NOs:1 and 3. The nucleotide sequences of SEQ ID NOs:1 and 3 are shown in FIGs. 12A-I and FIGs. 13A-H respectively. A nucleic acid sequence that is "substantially identical" to a reference nucleic acid sequence has a sequence that has at least 85% identity to the reference sequence, *e.g.*, the nucleic acid sequence of SEQ ID NOs:1 and 3. Of course, a substantially identical sequence can have a greater percentage of identity, *e.g.*, 90%, 95%, 96%, or 99% identity.

The nucleic acid molecules of the invention can be cDNA, genomic DNA, synthetic DNA, or RNA, and can be double-stranded or single-stranded (*i.e.*, either a sense or an antisense strand). Fragments of these molecules are also considered to be within the scope of the invention, and can be produced, for example, by the polymerase chain reaction (PCR) or generated by treatment with one or more restriction endonucleases. A ribonucleic acid (RNA) molecule can be produced by *in vitro* transcription.

The nucleic acid molecules of the invention can contain naturally occurring sequences, or sequences that differ from those that occur naturally, but, due to the degeneracy of the genetic code, encode the same polypeptide. In addition, these nucleic acid molecules are not limited to sequences that only encode polypeptides, and thus, can include some or all of the non-coding sequences that lie upstream or downstream from a coding sequence.

The nucleic acid molecules of the invention can be synthesized (for example, by phosphoramidite-based synthesis) or obtained from a biological cell. Combinations or modifications of the nucleotides within these types of nucleic acids are also encompassed.

In addition, the new isolated nucleic acid molecules encompass fragments that are not found as such in the natural state, but possess the same functions or uses as the full-length nucleic acids. The lengths of such fragments can range from very short to almost as long as the full-length nucleic acids of which they are fragments. For example, fragments of SEQ ID NOs:1 or 3 can range in length from 10 to 210 nucleic acids in length (*e.g.*, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, or 210 nucleic acids). Thus, the invention encompasses

recombinant molecules, such as those in which a nucleic acid molecule (for example, an isolated nucleic acid molecule such as SEQ ID NOs:1 or 3) is incorporated into a vector (for example, a plasmid or viral vector) or into the genome of a heterologous cell (or the genome of a homologous cell, at a position other than the natural chromosomal location). Recombinant nucleic acid molecules and uses therefor are discussed further below.

In the event the nucleic acid molecules of the invention encode or act as antisense molecules, they can be used for example, to regulate translation of mRNA of the invention. Given target sequences, techniques associated with detection or regulation of expression of those target nucleic acids or target polypeptides are known to skilled artisans and can be used to diagnose and/or treat disorders associated with aberrant expression of nucleic acids or polypeptides of the invention.

The invention also encompasses nucleic acid molecules that hybridize under stringent conditions to a nucleic acid molecule encoding a polypeptide of the invention. The cDNA sequences described herein can be used to identify these hybridizing nucleic acids, which include, for example, nucleic acids that encode homologous polypeptides in other species, and splice variants of the genes of the invention. Accordingly, the invention features methods of detecting and isolating these nucleic acid molecules. Using these methods, a sample (for example, a nucleic acid library, such as a cDNA or genomic library) is contacted (or "screened") with a probe specific to a nucleotide of the invention (for example, a fragment of SEQ ID NOs:1 or 3 that is at least 25 or 50 or 100 nucleotides long). The probe will selectively hybridize to nucleic acids encoding related polypeptides (or to complementary sequences thereof). The probe, which can contain at least 25 (for example, 25, 50, 100, 200, 300, or more than 300 nucleotides) can be produced using any of several standard methods (see, for example, Ausubel *et al.*, "Current Protocols in Molecular Biology, Vol. I," Green Publishing Associates, Inc., and John Wiley & Sons, Inc., NY, 1989). For example, the probe can be generated using PCR amplification methods in which oligonucleotide primers are used to amplify a nucleic acid sequence specific to a nucleic acid of the invention that can be used as a probe to screen a nucleic acid library and thereby detect nucleic acid molecules (within the library) that hybridize to the probe (*e.g.*, probes with nucleotide sequences complementary to nucleotides at positions 40-65, 120-170, 390-490, and 670-1170 of SEQ ID NO:1, or positions 65-136, 450-610, 1100-1650, or 4006-5009 of SEQ ID NO:3).

One single-stranded nucleic acid is said to hybridize to another if a duplex forms between them. This occurs when one nucleic acid contains a sequence that is the reverse and complement of

the other (this same arrangement gives rise to the natural interaction between the sense and antisense strands of DNA in the genome and underlies the configuration of the "double helix"). Complete complementarity between the hybridizing regions is not required for a duplex to form; it is only necessary that the number of paired bases is sufficient to maintain the duplex under the hybridization conditions used.

Typically, hybridization conditions are of low to moderate stringency. These conditions favor specific interactions between completely complementary sequences, but allow some non-specific interaction between less than perfectly matched sequences to occur as well. After hybridization, the nucleic acids can be "washed" under moderate or high conditions of stringency to dissociate duplexes that are bound together by some non-specific interaction (the nucleic acids that form these duplexes are thus not completely complementary).

As is known in the art, the optimal conditions for washing are determined empirically, often by gradually increasing the stringency. The parameters that can be changed to affect stringency include, primarily, temperature and salt concentration. In general, the lower the salt concentration and the higher the temperature, the higher the stringency. Washing can be initiated at a low temperature (for example, room temperature) using a solution containing a salt concentration that is equivalent to or lower than that of the hybridization solution. Subsequent washing can be carried out using progressively warmer solutions having the same salt concentration. As alternatives, the salt concentration can be lowered and the temperature maintained in the washing step, or the salt concentration can be lowered and the temperature increased. Additional parameters can also be altered. For example, use of a destabilizing agent, such as formamide, alters the stringency conditions.

In reactions where nucleic acids are hybridized, the conditions used to achieve a given level of stringency will vary. There is not one set of conditions, for example, that will allow duplexes to form between all nucleic acids that are 85% identical to one another; hybridization also depends on unique features of each nucleic acid. The length of the sequence, the composition of the sequence (for example, the content of purine-like nucleotides versus the content of pyrimidine-like nucleotides) and the type of nucleic acid (for example, DNA or RNA) affect hybridization. An additional consideration is whether one of the nucleic acids is immobilized (for example, on a filter).

An example of a progression from lower to higher stringency conditions is the following, where the salt content is given as the relative abundance of SSC (a salt solution containing sodium

chloride and sodium citrate; 2X SSC is 10-fold more concentrated than 0.2X SSC). Nucleic acids are hybridized at 42°C in 2X SSC/0.1% SDS (sodium dodecylsulfate; a detergent) and then washed in 0.2X SSC/0.1% SDS at room temperature (for conditions of low stringency); 0.2X SSC/0.1% SDS at 42°C (for conditions of moderate stringency); and 0.1X SSC at 68°C (for conditions of high stringency). Washing can be carried out using only one of the conditions given, or each of the conditions can be used (for example, washing for 10-15 minutes each in the order listed above). Any or all of the washes can be repeated. As mentioned above, optimal conditions will vary and can be determined empirically.

Once detected, the nucleic acid molecules can be isolated by any of a number of standard techniques (see, for example, Sambrook *et al.*, "Molecular Cloning, A Laboratory Manual," 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

The invention also encompasses: (a) expression vectors that contain any of the foregoing coding sequences (related to a polypeptide of the invention) and/or their complements (that is, "antisense" sequence); (b) expression vectors that contain any of the foregoing coding sequences (related to a polypeptide of the invention) operatively associated with a regulatory element (examples of which are given below) that directs the expression of the coding sequences; (c) expression vectors containing, in addition to sequences encoding a polypeptide of the invention, nucleic acid sequences that are unrelated to nucleic acid sequences encoding a polypeptide of the invention, such as molecules encoding a reporter or marker; and (d) genetically engineered host cells that contain any of the foregoing expression vectors and thereby express the nucleic acid molecules of the invention in the host cell.

The invention also encompasses heterologous fusions with endogenous human and murine genes. For example, many human oncogenes are hybrids comprised of a viral domain and a human domain. This suggests that, at some point, a virus integrated its sequence into a human, thus creating a chimeric sequence that is oncogenic.

Recombinant nucleic acid molecules can contain a sequence encoding a soluble polypeptide of the invention; mature polypeptide of the invention; or polypeptide of the invention having an added or endogenous signal sequence. A full-length polypeptide of the invention; a domain of a polypeptide of the invention; or a fragment thereof may be fused to additional polypeptides, as described below. Similarly, the nucleic acid molecules of the invention can encode the mature form of a polypeptide of the invention or a form that encodes a polypeptide that facilitates secretion. In

the latter instance, the polypeptide is typically referred to as a proprotein (or preprotein), which can be converted into an active form by removal of the signal sequence, for example, within the host cell. Proproteins can be converted into the active form of the protein by removal of the inactivating sequence.

The regulatory elements referred to above include, but are not limited to, inducible and non-inducible promoters, enhancers, operators and other elements, which are known to those skilled in the art, and which drive or otherwise regulate gene expression. Such regulatory elements include but are not limited to the cytomegalovirus hCMV immediate early gene, the early or late promoters of SV40 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major operator and promoter regions of phage A, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast α-mating factors.

Similarly, the nucleic acid can form part of a hybrid gene encoding additional polypeptide sequences, for example, sequences that function as a marker or reporter. Examples of marker or reporter genes include β-lactamase, chloramphenicol acetyltransferase (CAT), adenosine deaminase (ADA), aminoglycoside phosphotransferase (neo^r, $G418^r$), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding β-galactosidase), and xanthine guanine phosphoribosyltransferase (XGPRT). As with many of the standard procedures associated with the practice of the invention, skilled artisans will be aware of additional useful reagents, for example, of additional sequences that can serve the function of a marker or reporter. Generally, the hybrid polypeptide will include a first portion and a second portion; the first portion being a polypeptide of the invention and the second portion being, for example, the reporter described above or an immunoglobulin constant region.

The expression systems that may be used for purposes of the invention include, but are not limited to, microorganisms such as bacteria (for example, *E. coli* and *Bacillus subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA, or cosmid DNA expression vectors containing the nucleic acid molecules of the invention; yeast (for example, *Saccharomyces* and *Pichia*) transformed with recombinant yeast expression vectors containing the nucleic acid molecules of the invention (preferably containing the nucleic acid sequence encoding a polypeptide of the invention); insect cell systems infected with recombinant virus expression vectors (for example, baculovirus) containing the nucleic acid molecules of the invention; plant cell systems infected with recombinant

virus expression vectors (for example, cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (for example, Ti plasmid) containing nucleotide sequences of nucleic acids of the invention; or mammalian cell systems (for example, COS, CHO, BHK, 293, VERO, HeLa, MDCK, WI38, and NIH 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (for example, the metallothionein promoter) or from mammalian viruses (for example, the adenovirus late promoter and the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the gene product being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions containing polypeptides of the invention or for raising antibodies to those polypeptides, vectors that are capable of directing the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.* 2:1791, 1983), in which the coding sequence of the insert may be ligated individually into the vector in frame with the lacZ coding region so that a fusion protein is produced; pIN vectors (Inouye and Inouye, *Nucleic Acids Res.*, 13:3101-3109, 1985; Van Heeke and Schuster, *J. Biol. Chem.*, 264:5503-5509, 1989); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhidrosis virus (AcNPV) can be used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The coding sequence of the insert may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (for example, see Smith *et al.*, *J. Virol.*, 46:584, 1983; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the nucleic acid molecule of the invention may be ligated to an adenovirus transcription/translation control complex, for example, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (for example, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing a gene product of the invention in infected hosts (for example, see Logan and Shenk, *Proc. Natl. Acad. Sci. USA*, 81:3655-3659, 1984). Specific initiation signals may also be required for efficient translation of inserted nucleic acid molecules. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire gene or cDNA, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the coding sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner *et al.*, *Methods in Enzymol.*, 153:516-544, 1987).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (for example, glycosylation) and processing (for example, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells that possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. The mammalian cell types listed above are among those that could serve as suitable host cells.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines that stably express the sequences of nucleic acids or polypeptides of the

invention described above may be engineered. Rather than using expression vectors that contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (for example, promoter, enhancer sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci that in turn can be cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines that express nucleic acids or polypeptides of the invention. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that affect the endogenous activity of the gene product.

A number of selection systems can be used. For example, the herpes simplex virus thymidine kinase (Wigler, *et al.*, *Cell*, 11:223, 1977), hypoxanthine-guanine phosphoribosyltransferase (Szybalska and Szybalski, *Proc. Natl. Acad. Sci. USA*, 48:2026, 1962), and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell*, 22:817, 1980) genes can be employed in tk^-, $hgprt^-$ or $aprt^-$ cells, respectively. Also, anti-metabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler *et al.*, *Proc. Natl. Acad. Sci. USA*, 77:3567, 1980; O'Hare *et al.*, *Proc. Natl. Acad. Sci. USA*, 78:1527, 1981); gpt, which confers resistance to mycophenolic acid (Mulligan and Berg, *Proc. Natl. Acad. Sci. USA*, 78:2072, 1981); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin *et al.*, *J. Mol. Biol.*, 150:1, 1981); and hygro, which confers resistance to hygromycin (Santerre *et al.*, *Gene*, 30:147, 1984).

The nucleic acid molecules of the invention are useful in the diagnosis and treatment of systemic sclerosis and other autoimmune disorders.

Polypeptides

The invention also includes polypeptides that have a sequence that is encoded by, or is substantially identical to the polypeptides encoded by, the nucleic acids of the invention (*e.g.*, polypeptides that are substantially identical to a polypeptide encoded by either SEQ ID NO:1 or 3). The polypeptide sequences of centriolin (SEQ ID NO:2) and pericentrin-B (SEQ ID NO:4) are shown in FIGs. 12A-I and 13A-H, respectively. A polypeptide which is "substantially identical" to a

given reference polypeptide is a polypeptide having a sequence that has at least 85% identity to the sequence of the given reference polypeptide sequence (*e.g.*, the amino sequence of a polypeptide encoded either SEQ ID NO:1 or 3). Substantially identical polypeptides can also have a higher percentage identity, *e.g.*, 90%, 95%, 98%, or 99%.

The terms "protein" and "polypeptide" are used herein interchangably to describe any chain of amino acids, regardless of length or post-translational modification (for example, glycosylation or phosphorylation). Thus, the term "polypeptides of the invention" includes: full-length, naturally occurring proteins of the invention; recombinantly or synthetically produced polypeptides that correspond to full-length naturally occurring proteins of the invention; or particular domains or portions of the naturally occurring proteins. The term also encompasses mature polypeptides that have an added amino-terminal methionine (useful for expression in prokaryotic cells).

The centriolin and pericentrin-B polypeptides described herein are encoded by any of the nucleic acid molecules described herein and include SEQ ID NOs:2 and 4, as well as fragments, mutants, truncated forms, and fusion proteins of polypeptides of the invention. These polypeptides can be prepared for a variety of uses, including, but not limited to, the generation of antibodies, as reagents in diagnostic assays, for the identification of other cellular gene products or compounds that can modulate the activity or expression of nucleic acids or polypeptides of the invention, and as pharmaceutical reagents useful for the treatment of disorders associated with aberrant expression or activity of nucleic acids or polypeptides of the invention.

Useful polypeptides are substantially pure polypeptides of the invention, including those that correspond to the polypeptide with an intact signal sequence, and the secreted form of the polypeptide. Especially useful polypeptides are soluble under normal physiological conditions.

The invention also encompasses polypeptides that are functionally equivalent to polypeptides of SEQ ID NOs:2 and 4. These polypeptides are functionally equivalent to polypeptides of SEQ ID NOs:2 and 4 in that they are capable of carrying out one or more of the functions of polypeptides of the invention in a biological system. Useful polypeptides of the invention have 60%, 75%, 80%, or even 90% of one or more of the biological activities of the full-length polypeptides of SEQ ID NOs:2 and 4. Such comparisons are generally based on an assay of biological activity in which equal concentrations of the polypeptides are used and compared. The comparison can also be based on the amount of the polypeptide required to reach 50% of the maximal stimulation obtainable.

Functionally equivalent polypeptides can be those, for example, that contain additional or substituted amino acid residues. Substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, a functionally equivalent polypeptide is one in which 10% or fewer of the amino acids in a full-length, naturally occurring polypeptide are replaced by conservative amino acid substitutions, and the functionally equivalent polypeptide maintains at least 50% of the biological activity of the full-length polypeptide. Conservative amino acid substitution refers to the substitution of one amino acid for another amino acid of the same class (*e.g.*, valine for glycine and arginine for lysine).

Polypeptides that are functionally equivalent to polypeptides of SEQ ID NOs:2 and 4 can be made using random mutagenesis on the encoding nucleic acids by techniques well known to those skilled in the art. It is more likely, however, that such polypeptides will be generated by site-directed mutagenesis (again using techniques well known to those skilled in the art). These polypeptides may have increased functionality or decreased functionality.

To design functionally equivalent polypeptides, it is useful to distinguish between conserved positions and variable positions. This can be done by aligning the amino acid sequence of a protein of the invention from one species with its homolog from another species. Skilled artisans will recognize that conserved amino acid residues are more likely to be necessary for preservation of function. Thus, it is preferable that conserved residues are not altered.

Mutations within the coding sequence of nucleic acid molecules of the invention can be made to generate variant genes that are better suited for expression in a selected host cell. For example, N-linked glycosylation sites can be altered or eliminated to achieve, for example, expression of a homogeneous product that is more easily recovered and purified from yeast hosts that are known to hyperglycosylate N-linked sites. To this end, a variety of amino acid substitutions at one or both of the first or third amino acid positions of any one or more of the glycosylation recognition sequences which occur, and/or an amino acid deletion at the second position of any one or more of such recognition sequences, will prevent glycosylation at the modified tripeptide sequence (see, for example, Miyajima *et al.*, *EMBO J.*, 5:1193, 1986).

The polypeptides of SEQ ID NOs:2 and 4 can be expressed fused to another polypeptide, for example, a marker polypeptide or fusion partner. For example, the polypeptide can be fused to a

hexa-histidine tag to facilitate purification of bacterially expressed protein or a hemagglutinin tag to facilitate purification of protein expressed in eukaryotic cells.

A fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht *et al.* allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (*Proc. Natl. Acad. Sci. USA*, 88: 8972-8976, 1991). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni^{2+} . nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

The polypeptides of SEQ ID NOs:2 and 4 can be chemically synthesized (for example, see Creighton, "Proteins: Structures and Molecular Principles," W.H. Freeman & Co., NY, 1983), or, perhaps more advantageously, produced by recombinant DNA technology as described herein. For additional guidance, skilled artisans may consult Ausubel *et al.* (*supra*), Sambrook *et al.* ("Molecular Cloning, A Laboratory Manual," Cold Spring Harbor Press, Cold Spring Harbor, NY, 1989), and, particularly for examples of chemical synthesis Gait, M.J. Ed. ("Oligonucleotide Synthesis," IRL Press, Oxford, 1984).

The invention also features polypeptides that interact with nucleic acids or polypeptides of the invention (and the genes that encode them) and thereby alter the function of nucleic acids or polypeptides of the invention. Interacting polypeptides can be identified using methods known to those skilled in the art. One suitable method is the "two-hybrid system," which detects protein interactions *in vivo* (Chien *et al.*, *Proc. Natl. Acad. Sci. USA*, 88:9578, 1991). A kit for practicing this method is available from Clontech (Palo Alto, CA).

The invention also encompasses small polypeptides that can be used to inhibit specific interactions of two proteins (*e.g.*, a viral membrane protein and a host cell receptor). By virtue of their size, small polypeptides can have many useful properties. For example, they can be membrane-permeable if necessary, or they can be used at high concentration because of their specificity. Small polypeptides can also be used to generate very specific antibodies.

Antibodies

Polypeptides of the invention (or immunogenic fragments or analogs thereof) can be used to raise antibodies useful in the invention; such polypeptides can be produced by recombinant techniques or synthesized (see, for example, "Solid Phase Peptide Synthesis," *supra*; Ausubel *et al.*, *supra*). In general, the peptides can be coupled to a carrier protein, such as KLH, as described in Ausubel *et al.*, *supra*, mixed with an adjuvant, and injected into a host mammal. Antibodies can be purified by peptide antigen affinity chromatography.

In particular, various host animals can be immunized by injection with a polypeptide of the invention. Host animals include rabbits, mice, guinea pigs, and rats. Various adjuvants that can be used to increase the immunological response depend on the host species and include Freund's adjuvant (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. Useful human adjuvants include BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Polyclonal antibodies are heterogeneous populations of antibody molecules that are contained in the sera of the immunized animals.

Antibodies within the invention include polyclonal antibodies and, in addition, monoclonal antibodies, humanized or chimeric antibodies, single chain antibodies, Fab fragments, $F(ab')_2$ fragments, and molecules produced using a Fab expression library.

Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, can be prepared using the polypeptides of the invention described above and standard hybridoma technology (see, for example, Kohler *et al.*, *Nature*, 256:495, 1975; Kohler *et al.*, *Eur. J. Immunol.*, 6:511, 1976; Kohler *et al.*, *Eur. J. Immunol.*, 6:292, 1976; Hammerling *et al.*, "Monoclonal Antibodies and T Cell Hybridomas," Elsevier, NY, 1981; Ausubel *et al.*, *supra*).

In particular, monoclonal antibodies can be obtained by any technique that provides for the production of antibody molecules by continuous cell lines in culture such as described in Kohler *et al.*, *Nature*, 256:495, 1975, and U.S. Patent No. 4,376,110; the human B-cell hybridoma technique (Kosbor *et al.*, *Immunology Today*, 4:72, 1983; Cole *et al.*, *Proc. Natl. Acad. Sci. USA*, 80:2026, 1983), and the EBV-hybridoma technique (Cole *et al.*, "Monoclonal Antibodies and Cancer Therapy," Alan R. Liss, Inc., pp. 77-96, 1983). Such antibodies can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of

this invention may be cultivated *in vitro* or *in vivo*. The ability to produce high titers of mAbs *in vivo* makes this a particularly useful method of production.

Once produced, polyclonal or monoclonal antibodies are tested for specific recognition of polypeptides of the invention by Western blot or immunoprecipitation analysis by standard methods, *e.g.*, as described in Ausubel *et al.*, *supra*. Antibodies that specifically recognize and bind to polypeptides of the invention are useful in the invention. For example, such antibodies can be used in an immunoassay to monitor the level of a polypeptide of the invention produced by a mammal (for example, to determine the amount or subcellular location of a polypeptide of the invention).

Preferably, antibodies of the invention are produced using fragments of the protein of the invention that lie outside highly conserved regions and appear likely to be antigenic, by criteria such as high frequency of charged residues. In one specific example, such fragments are generated by standard techniques of PCR, and are then cloned into the pGEX expression vector (Ausubel *et al.*, *supra*). Fusion proteins are expressed in *E. coli* and purified using a glutathione agarose affinity matrix as described in Ausubel, *et al.*, *supra*.

In some cases it may be desirable to minimize the potential problems of low affinity or specificity of antisera. In such circumstances, two or three fusions can be generated for each protein, and each fusion can be injected into at least two rabbits. Antisera can be raised by injections in a series, preferably including at least three booster injections.

Antisera may also be checked for its ability to immunoprecipitate recombinant proteins of the invention or control proteins, such as glucocorticoid receptor, CAT, or luciferase.

In addition to their therapeutic uses, the new antibodies can be used, for example, in the detection of the polypeptide of the invention in a biological sample as part of a diagnostic assay. Antibodies also can be used in a screening assay to measure the effect of a candidate compound on expression or localization of a polypeptide of the invention. Additionally, such antibodies can be used in conjunction with the gene therapy techniques described to, for example, evaluate normal and/or genetically engineered cells that express nucleic acids or polypeptides of the invention prior to their introduction into the patient. Such antibodies additionally can be used in a method for inhibiting abnormal activity of nucleic acids or polypeptides of the invention.

In other embodiments, techniques developed for the production of "chimeric antibodies" (Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851, 1984; Neuberger *et al.*, *Nature*, 312:604, 1984; Takeda *et al.*, *Nature*, 314:452, 1984) by splicing the genes from a mouse antibody molecule

of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region.

Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies. Accordingly, lower dosages and less frequent administration are often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration (*e.g.*, into the brain). A method for lipidation of antibodies is described by Cruikshank *et al.* (*J. Acquired Immune Deficiency Syndromes and Human Retrovirology*, 14:193, 1997).

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent Nos. 4,946,778, 4,946,778, and 4,704,692) can be adapted to produce single chain antibodies against polypeptides of the invention. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide.

Antibody fragments that recognize and bind to specific epitopes can be generated by known techniques. For example, such fragments include but are not limited to $F(ab')_2$ fragments that can be produced by pepsin digestion of the antibody molecule, and Fab fragments that can be generated by reducing the disulfide bridges of $F(ab')_2$ fragments. Alternatively, Fab expression libraries can be constructed (Huse *et al.*, *Science*, 246:1275, 1989) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Antibodies to polypeptides of the invention can, in turn, be used to generate anti-idiotype antibodies that resemble a portion of the protein of the invention using techniques well known to those skilled in the art (see, *e.g.*, Greenspan et al., *FASEB J.*, 7:437, 1993; Nissinoff, *J. Immunol.*, 147:2429, 1991). For example, antibodies that bind to the protein of the invention and competitively inhibit the binding of a binding partner of the protein can be used to generate anti-idiotypes that resemble a binding partner binding domain of the protein and, therefore, bind and neutralize a binding partner of the protein. Such neutralizing anti-idiotypic antibodies or Fab fragments of such anti-idiotypic antibodies can be used in therapeutic regimens.

Antibodies can be humanized by methods known in the art. For example, monoclonal antibodies with a desired binding specificity can be commercially humanized (Scotgene, Scotland; Oxford Molecular, Palo Alto, CA). Fully human antibodies, such as those expressed in transgenic

animals are also features of the invention (Green *et al.*, *Nature Genetics*, 7:13-21, 1994; see also U.S. Patents 5,545,806 and 5,569,825).

The methods described herein in which anti-polypeptide-of-the-invention antibodies are employed may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one specific polypeptide-of-the-invention antibody reagent described herein, which may be conveniently used, for example, in clinical settings, to diagnose patients exhibiting symptoms of disorders associated with aberrant expression of nucleic acids or polypeptides of the invention.

An antibody (or fragment thereof) can be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent, or a radioactive agent (*e.g.*, a radioactive metal ion). Cytotoxins and cytotoxic agents include any agent that is detrimental to cells. Examples of such agents include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, and 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin {formerly designated daunomycin} and doxorubicin), antibiotics (*e.g.*, dactinomycin {formerly designated actinomycin}, bleomycin, mithramycin, and anthramycin), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

Conjugated antibodies (*i.e.*, antibodies joined to a moiety of a drug molecule) of the invention can be used for modifying a given biological response. The conjugated drug moiety need not be limited to classical chemical therapeutic agents. For example, the drug moiety can be a protein or polypeptide possessing a desired biological activity. Such proteins include, for example, toxins such as abrin, ricin A, *Pseudomonas* exotoxin, or *Diphtheria* toxin; proteins such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; and biological response modifiers such as lymphokines, interleukin-1, interleukin-2, interleukin-6, granulocyte macrophage colony stimulating factor, granulocyte colony stimulating factor, or other growth factors.

Techniques for conjugating a therapeutic moiety to an antibody are well known (see, *e.g.*, Arnon *et al.*, 1985, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", *in* Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.*, Eds., Alan R. Liss, Inc. pp. 243-256; Hellstrom *et al.*, 1987, "Antibodies For Drug Delivery", *in* Controlled Drug Delivery, 2nd ed., Robinson *et al.*, Eds., Marcel Dekker, Inc., pp. 623-653; Thorpe, 1985, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", *in* Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.*, Eds., pp. 475-506; "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", *in* Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al., Eds., Academic Press, pp. 303-316, 1985; and Thorpe *et al.*, 1982, Immunol. Rev., 62:119-158). Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

Antisense Nucleic Acids

Treatment regimes based on an "antisense" approach involve the design of oligonucleotides (either DNA or RNA) that are complementary to mRNA complementary to DNA sequences of the invention (*e.g.*, SEQ ID NO:1 or 3, or portions of these sequences). These oligonucleotides bind to the complementary mRNA transcripts of the invention and prevent translation. Absolute complementarity, although preferred, is not required. A sequence "complementary" to a portion of an RNA, as referred to herein, means a sequence having sufficient complementarily to be able to hybridize with the RNA *in vivo*, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarily and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs recently have been shown to be effective at inhibiting translation of mRNAs as well

(Wagner, *Nature*, 372:333, 1984). Thus, oligonucleotides complementary to either the 5' or 3' non-translated, non-coding regions of the gene or mRNA could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon.

Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5', 3', or coding region of an mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides, or at least 50 nucleotides.

Regardless of the choice of target sequence, it is preferred that *in vitro* studies are first performed to quantitate the ability of the antisense oligonucleotide to inhibit gene expression. It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligonucleotides. It is also preferred that these studies compare levels of the target RNA or protein with that of an internal control RNA or protein. Additionally, it is envisioned that results obtained using the antisense oligonucleotide are compared with those obtained using a control oligonucleotide. It is preferred that the control oligonucleotide is of approximately the same length as the test oligonucleotide and that the nucleotide sequence of the oligonucleotide differs from the antisense sequence no more than is necessary to prevent specific hybridization to the target sequence.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (as described, *e.g.*, in Letsinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 86:6553, 1989; Lemaitre *et al.*, *Proc. Natl. Acad. Sci. USA*, 84:648, 1987; PCT Publication No. WO 88/09810) or the blood-brain barrier (see, for example, PCT Publication No. WO 89/10134), or hybridization-triggered cleavage agents (see, for example, Krol *et al.*, *BioTechniques*, 6:958, 1988), or intercalating agents (see, for example, Zon, *Pharm. Res.*, 5:539, 1988). To this end, the

oligonucleotide can be conjugated to another molecule, for example, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethyl-aminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-theouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 2-(3-amino-3-N-2-carboxypropl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal, or an analog of any of these backbones.

In yet another embodiment, the antisense oligonucleotide is an α-anomeric oligonucleotide. An α-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gautier *et al.*, *Nucl. Acids. Res.*, 15:6625, 1987). The oligonucleotide is a 2'-0-methylribonucleotide (Inoue *et al.*, *Nucl. Acids Res.*, 15:6131, 1987), or a chimeric RNA-DNA analog (Inoue *et al.*, *FEBS Lett.*, 215:327, 1987).

Antisense oligonucleotides of the invention can be synthesized by standard methods known in the art, *e.g.*, by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides can be synthesized by the method of Stein *et al.* (*Nucl. Acids Res.*, 16:3209, 1988), and methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin *et al.*,

Proc. Natl. Acad. Sci. USA, 85:7448, 1988). Examples of antisense molecules that can be used in the methods of the invention include RNA molecules identical to the sequences stretching from nucleotide positions 101-203, 509-725, 2002-2310, and 3907-4702 of SEQ ID NO:1 or positions 567-734, 2346-2678, 3006-3123, and 3634-4201 of SEQ ID NO:3, with U's substituted for all T's. Many other such examples of antisense molecules are also available.

The antisense molecules should be delivered to cells that express nucleic acids or polypeptides of the invention *in vivo*. A number of methods have been developed for delivering antisense DNA or RNA to cells; *e.g.*, antisense molecules can be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (*e.g.*, antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

However, it is often difficult to achieve intracellular concentrations of the antisense molecule sufficient to suppress translation of endogenous mRNAs. Therefore, a preferred approach uses a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong *pol* III or *pol* II promoter. The use of such a construct to transfect target cells in the patient will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous transcripts of nucleic acids of the invention and thereby prevent translation of the endogenous mRNA. The invention encompasses the construction of an antisense RNA using the complementary strand as a template. For example, a vector can be introduced *in vivo* such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA.

Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to: the SV40 early promoter region (Bernoist *et al.*, *Nature* 290:304, 1981); the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto *et al.*, *Cell*, 22:787-797, 1988); the herpes thymidine kinase promoter (Wagner *et al.*, *Proc. Natl. Acad. Sci. USA*, 78:1441, 1981); or the regulatory sequences of the metallothionein gene (Brinster *et al.*, *Nature*, 296:39, 1988).

Gene Silencing

Double-stranded nucleic acid molecules can be used to silence or inhibit expression of a gene of the invention (*e.g.*, SEQ ID NO:1 or 3). RNA interference (RNAi) is a mechanism of post-transcriptional gene silencing in which double-stranded RNA (dsRNA) corresponding to a gene (or coding region) of interest is introduced into a cell or an organism, resulting in degradation of the corresponding mRNA. The RNAi effect persists for multiple cell divisions before gene expression is regained. RNAi is therefore an extremely powerful method for making targeted knockouts or "knockdowns" at the RNA level. RNAi has proven successful in human cells, including human embryonic kidney and HeLa cells (see, *e.g.*, Elbashir *et al.*, *Nature*, 411:494-8, 2001). In one embodiment, gene silencing can be induced in mammalian cells by enforcing endogenous expression of RNA hairpins (see Paddison *et al.*, *PNAS*, 99:1443-1448, PNAS). In another embodiment, transfection of small (21-23 nt) dsRNA specifically inhibits gene expression (reviewed in Caplen, *Trends in Biotechnology*, 20:49-51, 2002).

Briefly, RNAi is thought to work as follows. dsRNA corresponding to a portion of a gene to be silenced is introduced into a cell. The dsRNA is digested into 21-23 nucleotide siRNAs, or short interfering RNAs. The siRNA duplexes bind to a nuclease complex to form what is known as the RNA-induced silencing complex, or RISC. The RISC targets the homologous transcript by base pairing interactions between one of the siRNA strands and the endogenous mRNA. It then cleaves the mRNA ~12 nucleotides from the 3' terminus of the siRNA (reviewed in Sharp *et al.*, *Genes Dev*, 15:485-490, 2001; Hammond *et al.*, *Nature Rev Gen*, 2:110-119, 2001).

RNAi technology in gene silencing utilizes standard molecular biology methods. dsRNA corresponding to the sequence from a target gene to be inactivated can be produced by standard methods, *e.g.*, by simultaneous transcription of both strands of a template DNA (corresponding to the target sequence) with T7 RNA polymerase. Kits for production of dsRNA for use in RNAi are available commercially (*e.g.*, from New England Biolabs, Inc.). Methods of transfection of dsRNA or plasmids engineered to make dsRNA are routine in the art.

Gene silencing effects similar to those of RNAi have been reported in mammalian cells with transfection of a mRNA-cDNA hybrid construct (Lin *et al.*, *Biochem Biophys Res Commun*, 281(3):639-44, 2001), providing yet another strategy for gene silencing.

SiRNA sequences to centriolin and pericentrin-B can be designed, for example, using siDESIGN Center or the Custom SMARTPool siRNA Design Service (Dharmacon Research, Inc.).

Centriolin siRNA sequences that can be used in the invention include the following (all listed from 5' to 3': GGAUCAGAGACUCUACCUU (SEQ ID NO:8), GCUGAUUCACAUGCAGGAG (SEQ ID NO:9), GACGAGGCUAUUGGUACUU (SEQ ID NO:10), AAGCAAAGAUACCAUCAUC (SEQ ID NO:11), GUGGUGUGAGCAAAUUGAG (SEQ ID NO:12), AGACCAUAAAGGAGCUGAU (SEQ ID NO:13), GACCAUAAAGGAGCUGAUU (SEQ ID NO:14), and UUCACAUGCAGGAGUUAGA (SEQ ID NO:15). These are merely included as a representative sample of the many centriolin siRNA sequences that can be employed in the invention.

Pericentrin-B siRNA sequences that can be used in the invention include the following (all listed from 5' to 3': UUGGAACAGCUGCAGCAGA (SEQ ID NO:16), AGCUGAGCUGAAGGAGAAG (SEQ ID NO:17), GAAGGAGAAGGAGACGGCA (SEQ ID NO:18), AAAGGUGACAGUUCGCAUU (SEQ ID NO:19), CAGUUCGCAUUCGGAGAAA (SEQ ID NO:20), GCAGACUGUAGUGCGAGAU (SEQ ID NO:21), GCCGUGUCUAAGCUUGAGA (SEQ ID NO:22), and UCACAUCUCGUCCUUUCAC (SEQ ID NO:23). These are merely included as a representative sample of the many centriolin siRNA sequences that can be employed in the invention.

Ribozymes

Ribozyme molecules designed to catalytically cleave mRNA transcripts of nucleic acids of the invention (*e.g.*, SEQ ID NOs:1 or 3 depicted in FIGs. 12A-I and 13A-H, respectively) can be used to prevent translation and expression of mRNA of the invention. (see, *e.g.*, PCT Publication WO 90/11364; Saraver *et al.*, *Science*, 247:1222, 1990). While various ribozymes that cleave mRNA at site-specific recognition sequences can be used to destroy mRNAs of the invention, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art (Haseloff *et al.*, *Nature*, 334:585, 1988). Preferably, the ribozyme is engineered so that the cleavage recognition site

is located near the 5' end of the mRNA, i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes"), such as the one that occurs naturally in *Tetrahymena thermophila* (known as the IVS or L-19 IVS RNA), and which has been extensively described by Cech and his collaborators (Zaug *et al.*, *Science*, 224:574, 1984; Zaug *et al.*, *Science*, 231:470, 1986; Zug *et al.*, *Nature*, 324:429, 1986; PCT Application No. WO 88/04300; and Been *et al.*, *Cell*, 47:207, 1986). The Cech-type ribozymes have an eight base-pair sequence that hybridizes to a target RNA sequence, whereafter cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes that target eight base-pair active site sequences present in nucleic acids of the invention.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (e.g., for improved stability, targeting, etc.), and should be delivered to cells that express nucleic acids or polypeptides of the invention *in vivo*. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive *pol* III or *pol* II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous messages and inhibit translation. Because ribozymes, unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Screening Assays

Compounds with unknown function can be screened to determine whether they specifically bind to nucleic acids or polypeptides of the invention using any standard binding assay. For example, candidate compounds can be bound to a solid support. A nucleic acid or polypeptide of the invention is then exposed to the immobilized compound and binding is measured (*e.g.*, as done in European Patent Application 84/03564).

In one embodiment, the invention provides assays for screening candidate or test compounds that bind with or modulate the activity of the membrane-bound form of a polypeptide of the invention or biologically active portion thereof. The test, or "candidate", compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound"

library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer, or small molecule libraries of compounds (Lam, *Anticancer Drug Des.*, 12:145, 1997).

Examples of methods useful for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6909, 1993; Erb *et al.*, *Proc. Natl. Acad. Sci. USA*, 91:11422, 1994; Zuckermann *et al.*, *J. Med. Chem.*, 37:2678, 1994; Cho *et al.*, *Science*, 261:1303, 1993; Carrell *et al.*, *Angew. Chem. Int. Ed. Engl.*, 33:2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl.*, 33:2061, 1994; and Gallop *et al.*, *J. Med. Chem.*, 37:1233. 1994.

Libraries of compounds can be presented in solution (*e.g.*, Houghten, *Bio/Techniques*, 13:412-421, 1992), or on beads (Lam, *Nature*, 354:82-84, 1991), chips (Fodor, *Nature*, 364:555-556, 1993), bacteria (U.S. Patent No. 5,223,409), spores (U.S. Patent Nos. 5,571,698; 5,403,484; and 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:1865-1869, 1992) or phage (Scott and Smith, *Science*, 249:386-390, 1990; Devlin, *Science*, 249:404-406, 1990; Cwirla *et al.*, *Proc. Natl. Acad. Sci. USA*, 87:6378-6382, 1990; and Felici, *J. Mol. Biol.*, 222:301-310, 1991).

In one embodiment, an assay is a cell-based assay in which a cell that expresses a membrane-bound form of a polypeptide of the invention, or a biologically active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind with the polypeptide is determined. The cell, for example, can be a yeast cell or a cell of mammalian origin. Determining the ability of the test compound to bind with the polypeptide can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the polypeptide or biologically active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radio-emission or by scintillation counting. Alternatively, test compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of a polypeptide of the invention, or a biologically active portion thereof, on the cell surface with a known compound that binds the polypeptide to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the

polypeptide, wherein determining the ability of the test compound to interact with the polypeptide comprises determining the ability of the test compound to preferentially bind with the polypeptide or a biologically active portion thereof as compared to the known compound.

In another embodiment, the assay involves assessment of an activity characteristic of the polypeptide, wherein binding of the test compound with the polypeptide or a biologically active portion thereof alters (i.e., increases or decreases) the activity of the polypeptide.

Methods of Use

The invention provides the nucleic acid and polypeptide sequences of centriolin and pericentrin-B. These nucleic acid sequences themselves have many uses, for example, in the production of hybridization probes to locate identical, homologous, or similar DNA or RNA sequences, within either humans or other organisms (especially mammals).

The invention includes methods of reducing or preventing cell division by, for example, blocking the replication, expression, or translation of centriolin or pericentrin-B, or by, for example, interfering with the activity of centriolin or pericentrin-B polypeptide. Abnormalities in cell division have been linked to numerous debilitating diseases and disorders, including cell proliferation disorders, not the least of which are cancers, leukemia, psoriasis, and leukemia. Because of their pivotal role in modulating cell division, centriolin and pericentrin-B can be used to treat such diseases and disorders at the subcellular level of causation. For example, methods of the invention can be used to treat cancer by modulating centriolin or pericentrin-B (*e.g.*, via RNAi, siRNA, antisense nucleic acids, ribozymes, antibodies) so as to drive cancer cells into cell-cycle arrest. For example, the siRNA molecules of SEQ ID NOs:8, 9, and 10 can be used effectively to arrest the cell-cycle by interfering with centriolin. In a patient with a cell proliferative disorder (*e.g.*, psoriasis, cancers, leukemia, Hodgkin's disease, lymphomas, myelofibrosis, polycythemia vera), these molecules can be delivered to the patient to stop cell proliferation, thereby ameliorating the symptoms of the disease and slowing or stopping its progression. Similarly, siRNA molecules of SEQ ID NOs:16, 17, and 18 can be used effectively to arrest the cell-cycle by interfering with pericentrin-B. Such molecules can also be delivered to particular tissues most affected by unhealthy cell proliferation, thus localizing cell-cycle arrest.

In addition, the invention provides methods of restoring normal centriolin or pericentrin-B function (*e.g.*, to cells in which centriolin or pericentrin-B expression or activity is inadequate

to sustain a normal cell division cycle) by administering therapeutically effective amounts of centriolin or pericentrin-B polypeptides. This provides a method of treatment for debilitating diseases in which cell proliferation is inadequate for healthy bodily functioning (e.g., in healing of wounds or replacement of epithelial cells).

The invention also provides a method for the diagnosis of abnormal centriolin and pericentrin-B expression or activity, thus allowing, for example, valuable insight into the etiology of diseases, disorders, or symptoms thereof that have previously defied medically useful explanation. Having linked any such disease, disorder, or symptom thereof to abnormal centriolin and pericentrin-B expression or activity, methods of the invention then provide treatments heretofore unavailable or unknown.

Effective Dose

Toxicity and therapeutic efficacy of the molecules disclosed in the invention (*e.g.*, nucleic acids, polypeptides, ribozymes, antibodies *etc.*) and the compounds that modulate their expression or activity can be determined by standard pharmaceutical procedures, using either cells in culture or experimental animals to determine the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Polypeptides or other compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (that is, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture.

Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

Formulations and Use

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

Thus, the compounds and their physiologically acceptable salts and solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral or rectal administration.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (for example, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (for example, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (for example, magnesium stearate, talc or silica); disintegrants (for example, potato starch or sodium starch glycolate); or wetting agents (for example, sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (for example, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (for example, lecithin or acacia); non-aqueous vehicles (for example, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (for example, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, for example, dichlorodifluoromethane,

trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, for example, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, for example, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, for example, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, for example, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, for example, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The compositions may, if desired, be presented in a pack or dispenser device that may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

The therapeutic compositions of the invention can also contain a carrier or excipient, many of which are known to skilled artisans. Excipients that can be used include buffers (for example, citrate buffer, phosphate buffer, acetate buffer, and bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid, phospholipids, proteins (for example, serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol. The nucleic acids, polypeptides, antibodies, or modulatory compounds of the invention can be administered by any standard route of

administration. For example, administration can be parenteral, intravenous, subcutaneous, intramuscular, intracranial, intraorbital, opthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, transmucosal, or oral. The modulatory compound can be formulated in various ways, according to the corresponding route of administration. For example, liquid solutions can be made for ingestion or injection; gels or powders can be made for ingestion, inhalation, or topical application. Methods for making such formulations are well known and can be found in, for example, "Remington's Pharmaceutical Sciences". It is expected that the preferred route of administration will be intravenous.

It is recognized that the pharmaceutical compositions and methods described herein can be used independently or in combination with one another. That is, subjects can be administered one or more of the pharmaceutical compositions, e.g., pharmaceutical compositions comprising a nucleic acid molecule or protein of the invention or a modulator thereof, subjected to one or more of the therapeutic methods described herein, or both, in temporally overlapping or non-overlapping regimens. When therapies overlap temporally, the therapies may generally occur in any order and can be simultaneous (e.g., administered simultaneously together in a composite composition or simultaneously but as separate compositions) or interspersed. By way of example, a subject afflicted with a disorder described herein can be simultaneously or sequentially administered both a cytotoxic agent which selectively kills aberrant cells and an antibody (e.g., an antibody of the invention) which can, in one embodiment, be conjugated or linked with a therapeutic agent, a cytotoxic agent, an imaging agent, or the like.

EXAMPLES

The invention is further described in the following examples, which do not limit the scope of the invention described in the claims. The general experimental procedures are described first.

Experimental Procedures

Western Blotting

Western blotting was carried out in centrosome fractions prepared from HeLa cells. The IF method was employed. Embryos were processed as described in Doxsey *et al.* (*Cell*, 76:639-650, 1994). RT-PCR bands were sequenced.

Cell Culture and Transfections

The cells used primarily in this study were diploid, telomerase-immortalized human retinal pigment epithelial cells (hTERT-RPE1s, Clontech) (Morales *et al., Nat Genet,* 21:115-118, 1999). Other cells included HeLa, COS-7, hTERT-HME1 (human mammary epithelia), Saos, U2OS and *Xenopus* tissue culture cells (XTC). All were grown as described (American Type Culture Collection). COS-7 cells were transfected with cDNAs encoding centriolin, the Nud1 domain, and the pericentrin-B centrosome localization domain as described (Lipofectamine, InVitrogen).

Antibody Production

Amino acids 268-903 or centriolin were fused with glutathione-S-transferase (GST, Clontech), overexpressed in E. coli and purified as described (Doxsey *et al., Cell,* 76:639-650, 1994). Antibodies raised in rabbits were affinity-purified by passing sera over a GST column to remove anti-GST antibodies then over a GST-centriolin column. Antibodies to the following proteins were also used in this study: lamin A/C (Cell Signaling Technology), a and g tubulins, (Sigma), LexA (Santa Cruz), GAL4 TAD (Clontech), pericentrin-B (T. Davis, R. Balczon), Ki-67, hemagglutinin (BD Biosciences).

Aster Formation Assays

Xenopus mitotic egg extracts were obtained as described in Doxsey (1994 #131). Asters were formed by adding 1ul of sperm (104) to 8.8 µl of crude egg extract containing 0.2ul of 10mg/ml rhodamine tubulin (Cytoskeleton). This mixture was incubated at 25° C for 7 minutes then affinity-purified centriolin antibody or rabbit IgG (Sigma) were added to reach a final concentration of 50ug/ml. At 0, 5, 10 and 15 minutes after antibody addition, samples were prepared as described in Doxsey (1994), and immediately viewed for the presence of asters under a Zeiss Axiophot fluorescence microscope equipped with DAPI and rhodamine filters. The number of asters associated with sperm were recorded for each timepoint.

Immunofluorescence and Electron Microscopy

Cells were prepared for immunofluorescence and imaged as described (Dictenberg *et al.*, *J Cell Biol.*, 141:163-174, 1998) then deconvolved using Metamorph software (Universal Imaging Corp.). Immunogold electron microscopy was performed as described (Doxsey *et al.*, *Cell*, 76:639-650, 1994) using centrosome fractions from HeLa cells (Blomberg and Doxsey, *Meth Enzymol*, 298:228-238, 1998) and antibodies to centriolin followed by antibodies coupled to 5 nm gold particles (Amersham).

Centriolin and Pericentrin-B Cloning and Sequencing

A cDNA of ~1.7 kB was identified by screening a human placenta expression library with serum from individuals with scleroderma (Doxsey *et al.*, *Cell*, 76:639-650, 1994). The nucleotide sequence was compared with others (blastn) in the human genome database (NCBI) and revealed a sequence with 99% identity on chromosome 9 q34.11-34.13 (Genbank accession # AF513978). Genscan predicted a ~7 kb gene comprising 40 exons. PCR primers were used to obtain a ~7 Kb cDNA in a human testes cDNA library. The 5' end, obtained by rapid amplification of cDNA ends (RACE), was identical to the predicted sequence. A full-length HA-tagged centriolin was obtained by inserting an HA tag (YPYDVPDYASL) 5' to the RACE fragment and ligating the HA-centriolin cDNA to the original fragment. The full length centriolin cDNA contained 6,975 nucleotides with an ORF of 2,325 amino acids and predicted molecular mass of 269 kD, consistent with the molecular mass of endogenous centriolin.

Regions flanking the ORF had a translational start (Kozak sequence), polyadenylation sequence, poly-A tail and multiple upstream and downstream stop codons. The construct was inserted into pcDNA 3.1 Zeo (+) (InVitrogen) using BamHI and NotI restriction sites. Centriolin was translated in vitro (TNT, Promega) and expressed in cultured cells using conventional procedures (Lipofectamine, InVitrogen). Centriolin amino acids 435-623 and 1385-1658 were 24% identical/47% similar and 20% identical/41% similar to the COOH-terminal half of TACCs, respectively. Amino acids 879-913 were 40% identical/51% similar to amino acids 72-106 of human stathmin. Amino acids 126-234 were 31-35% identical/47%-50% similar to Nud1p and Cdc11p.

Using the cDNA sequence of the human pericentrin-B (GenBank accession numbers U52962 &XM_036857), the 10,011bp cDNA was cloned using a combination of library

screening and RT-PCR. A clone containing ~4kB of the 3' pericentrin-B sequence was isolated from a human lung cDNA library by screening with a short cDNA, and the remaining ~6kb was obtained by RT-PCR from human testis mRNA in 1 kB sections. An HA tag was added 5' to the start methionine and the resulting cDNA was cloned into the pcDNA3.1 vector between the Nhe I and Not I sites (Cytomyx, Cambridge UK). Both strands of the cDNA were sequenced.

siRNAs and Morpholino Antisense

siRNAs targeting centriolin, pericentrin-B, lamin A/C, and GFP mRNAs were made as complimentary single stranded 19-mer siRNAs with 3' dTdT overhangs (Dharmacon Research), deprotected, annealed and delivered into cells (Oligofectamine, Invitrogen). A 400 mM stock was used to increase the efficiency of gene silencing observed using published stocks. Nucleotides targeted: in centriolin, 117-136 (FIG. 4A-G) and 145-163, in pericentrin-B, 301-319 (FIG. 7A-G) and 975-993, in lamin A/C, 608-630 (Genbank Acc. No. X03444) and pEGFP-C1 (Clontech), 233-252.

Fluorescein-conjugated morpholino antisense DNA oligonucleotides (Gene Tools) targeting the start codon of centriolin (5'-TTTGTTGAGAACCTTTCTTCATTGC) were introduced into cells using the EPEI agent (Gene Tools). The inverse sequence was used as control.

Time-lapse Imaging

HeLa cells plated on coverslips (25 mm diameter) were treated with siRNAs targeting centriolin for 50 h. They were placed in a chamber (PDMI-2; Harvard Apparatus) in complete medium with CO_2 exchange (0.5 liters/min) at 37 °C. Cells were imaged every 10 min for 12-20 h using a 20x or 40x phase contrast lens with a green interference filter on an inverted microscope (Olympus IX-70). Images were captured on a CoolSnap HQ CCD camera (Roper Scientific) and concatenated using Metamorph software (Universal Imaging Corp.).

RT-PCR to Determine mRNA Levels Following siRNA Treatment

RNA was extracted and purified (Ologotex Direct mRNA miniKit, QIAGEN) and mRNA levels were assessed by the reverse transcription polymerase chain reaction (RT-PCR) with 10ml of mRNA using OneStep RT-PCR Kit (QIAGEN). Alpha tubulin was amplified in the same

tubes as the experimental sample to serve as an internal control. PCR products were subjected to electrophoresis in 1% agarose gel and stained with ethidium bromide. Product authenticity was confirmed by DNA sequencing. Primers used were for pericentrin-B, forward 5'-AACACTCTCCATGATTGCCC-3' and reverse 5'-TACCCTCCCAATCTTTGCTG-3' (GenBank Acc. No. XM_036857).

Cell Synchronization and Cell Cycle Analysis

hTERT-RPE1 cells treated with siRNAs and nocodazole (100 ng/ml) for 10 hrs, were washed free of drug and grown for various times before being prepared for immunofluorescence. To examine mitotic cells only, nocodazole-treated mitotic cells were released from plates, washed and re-plated on coverslips. After they adhered (~90 min) most were in telophase; FIG. 4A-G shows cells at the end of the wave of cytokinesis. Flow cytometry was performed on cells stained with propidium iodide (Sigma) using FACScan (Becton Dickinson) and FloJo software.

Primary Cilium Formation

Primary cilia were induced by culturing hTERT-RPE1 cells in medium with 0.25% serum for 48 hrs and identified using the GT335 antibody raised to polygluatamylated α- and β-tubulins (Bobinnec *et al., J Cell Biol.*, 143:1575-1589, 1998). Microtubule nucleation was performed as previously described (Purohit *et al., J. Cell Biol.*, 147:481-491, 1999) by treatment with nocodazole (1mg/ml) for 1 h at 37 °C, fixing cells at various times after washing out drug, and then staining for microtubules.

RT-PCR

Centriolin and pericentrin-B mRNA levels were assayed by reverse transcription polymerase chain reaction (RT-PCR) using 10 µl mRNA (OneStep RT-PCR, Qiagen); α-tubulin served as an internal control in the same reaction. Alls products were sequenced. Primers: specific for pericentrin-B (and not pericentrin-A), forward 5'-AACACTCTCCATGATTGCCC-3' and reverse 5'-TACCCTCCCAATCTTTGCTG-3' (GeneBank Acc. No. XM036857); for human a tubulin, forward 5'-AAAGATGTCAATGCTGCC-3' and reverse 5'-

TCCTCTCCTTCTTCCTCAC-3'; for centriolin, forward 5'-CCATCATCATCTCACTCTC-3' and reverse 5'-CTTCCCTAACCATACTGG-3'.

Yeast Two Hybrid Analysis and Immunoprecipitations

A 321 base pair fragment containing amino acids 127-233 of centriolin were cloned into EcoR I and Sal I sites of pGADT7 (Clontech) to produce a fusion with the GAL4 transactivation domain (TAD). Constructs pEG202 (LexA), pGP69 (LexA-BUB2), pGP122 (LexA-BFA1) and the yeast strain SGY37 were from Elmar Schiebel (Paterson Institute for Cancer Research, Manchester, U.K.). SGY37, which contains a LacZ reporter gene under control of a LexA operator, was transformed with plasmid DNA using LiAc (Ito *et al., J Bacteriol,* 153:163-168, 1983) and transformants selected for on dropout medium. Methods used were semi-quantitative β-galactosidase assays (Schramm *et al., Methods Cell Biol.,* 67:71-94, 2001) and more quantitative β-galactosidase assays with CPRG (chlorophenol red-b-D-galactopyranoside, Roche) as a substrate per the manufacturer's instructions (Clontech Yeast Protocols Handbook). Co-immunopreciptation of LexA and GAL4 TAD fusion proteins were carried out as previously described (Schramm *et al., Methods Cell Biol.,* 67:71-94, 2001).

Semi-quantitative β-galactosidase assays were performed by growing yeast on selective media and overlaying with a solution of 0.4% agarose, 145 mM Na2HPO4, 106 mM NaH2PO4 (pH7.0), 0.5% SDS, 10 mM KCl, 1 mM MgCl2, 0.4 mg/ml 5-bromo-4-chloro-indolyl-b-D galactopyranoside (Sigma) that had been allowed to cool to 40 °C. After the agarose had solidified the plates were incubated at 30 °C until a color change was observed.

Quantitative β-galactosidase assays were performed using chlorophenol red-b-D-galactopyranoside (CPRG) as a substrate. Transformed yeast were used to inoculate 2 ml of SD-L-H medium and grown overnight at 30 °C with shaking at 280 rpm. The cultures were then diluted 1:5 with YPDA and grown until the OD600 nm was between 0.5 and 0.8. A 3 ml volume of culture was removed, the cells pelleted by centrifugation at 14,000 rpm for 1 minute and the supernatant discarded. A single wash with CPRG assay buffer (0.05% Tween 20, 1% BSA, 100 mM HEPES, 154 mM NaCl, 4.5 mM L-aspartate) was carried out, the cells re-suspended in 0.6 ml CPRG assay buffer and aliquoted in 0.1 ml volumes into 1.5 ml Eppendorf tubes. The cells were lysed by repeatedly snap freezing in liquid nitrogen and thawing in a 37 °C water bath for a total of three times. A 0.7 ml volume of CPRG substrate buffer (2.23 mM CPRG, 0.05% Tween

20, 1% BSA, 100 mM HEPES, 154 mM NaCl, 4.5 mM L-aspartate) was added to each tube, the contents briefly mixed by vortexing and the samples incubated at 30 °C until a color change was observed. Reactions were stopped by adding 0.5 ml of 3 mM ZnCl2 and pelleting the cellular debris by centrifugation at 14,000 rpm for 2 minutes. The OD578 nm measured against a blank and used to calculate the number of β-galactosidase units produced.

Yeast cells expressing the indicated proteins either alone or in combination were used for immunoprecipitations. The hNud1-β-galactosidase fusion protein or β-galactosidase alone were immunoprecipitated using antibodies to β-galactosidase. LexA-Bub2p and LexA-Bfa1p fusion proteins and LexA alone were immunoprecipitated using an anti-LexA antibody. Following immunoprecipitation with one antibody, the other antibody was used to detect proteins that co-immunoprecipitate by Western blotting. Conditions and buffers for Ips were those specified in (Ito *et al., J. Bacteriol,* 153:163-168, 1983).

Flow Cytometry

Cells treated with siRNAs for 50-70 h were treated with 100 ng/ml for 12 h, removed from plates, and fixed in methanol. Cells stained with propidium iodide were analyzed for flow cytometry (FACSCAN®; Becton Dickinson) using Flojo software (Tree Star, Inc.).

GenBank/EMBL/DDBJ Accession Numbers

The GenBank/EMBL/DDBJ accession number for centriolin is AF513978. The accession number for lamin A/C is X03444. And, the accession numbers for pericentrin-B are U52962 and XM_036857.

Example 1. Identification and Cloning of a 260 kD Protein Localized to the Maternal Centriole and Intercellular Bridge

Using sera from patients with the autoimmune disease scleroderma that react with centrosomes (Doxsey *et al., Cell,* 76:639-650, 1994) a human placenta lgt11 cDNA expression library was screened to identify genes encoding the autoantigens. Of the 3 x 106 clones screened, only one of 1.7 Kb was identified indicating that the mRNA for this molecule was rare. The protein encoded by the cDNA was called centriolin. Antibodies raised against recombinant centriolin recognized a band of ~270 kD on Western blots of isolated centrosome fractions from

cells of a wide range of species including human and *Xenopus* (FIG. 1A); preimmune sera showed no specific bands. The protein was not detected by Western blotting of whole-cell lysates, consistent with the probable low abundance of this and other centrosome autoantigens (Doxsey *et al., Cell,* 76:639-650, 1994). *In vitro* translation and overexpression of the protein in mammalian cells using the full-length cDNA produced a protein with a molecular weight similar to the endogenous protein (FIG. 1A).

Immunofluorescence microscopy demonstrated that centriolin was localized to centrosomes in a wide variety of species including human, monkey, hamster, mouse, and *Xenopus* (FIGs. 1A-B and 2A-G). Centrosome localization was confirmed by showing that a hemagglutinin (HA)-tagged centriolin protein ectopically expressed in COS cells localized to centrosomes (FIG. 2A). The endogenous protein was present on the centrosome throughout the cell cycle. In late G1/early S phase centrosomes begin to duplicate and by G2/M duplication is completed. During the duplication process, centriolin was present on only one of the two duplicating centrosomes although other proteins such as g tubulin were found on both (FIG. 1B). Beginning at prometaphase, dim staining was observed next to a brightly-stained centrosome. By metaphase when centrosomes become "mature" (Lange and Gull, *J Cell Biol.,* 130:919-927, 1995), both centrosomes had equally high levels of centriolin and were higher than any other cell cycle stage. At the metaphase to anaphase transition, centriolin staining was rapidly reduced at centrosomes and reached its lowest levels by late anaphase/telophase. During cytokinesis, centriolin appeared at the intercellular bridge, initially as one or two dots adjacent to the bridge (FIG. 1B, Telo early) suggesting that the centrosome/centriole had moved to this site. The staining pattern was consistent with recent time lapse imaging experiments showing that the maternal centriole translocates to the intercellular bridge during cytokinesis (Piel *et al., Science,* 291:1550-1553, 2001). Centriolin next appeared as diffusely organized material within the intercellular bridge and then became concentrated at the midbody (FIG. 1B, Telo late).

The organization of centriolin at the centrosome was more precisely determined by serum-starving cells to induce growth of a primary cilium from the maternal centriole (Vorobjev and Chentsov, *J Cell Biol.,* 93:938-949, 1982). In these cells, centriolin staining was confined to the maternal centriole underlying the cilium (FIG. 2B). Immunogold electron microscopy on centrosome fractions (Blomberg and Doxsey, *Meth Enzymol,* 298:228-238, 1998; Doxsey *et al., Cell,* 76:639-650, 1994) confirmed localization to the maternal centriole (FIG. 2E) and further

demonstrated that the protein was concentrated on subdistal appendages, specialized substructures of the maternal centriole implicated in microtubule anchoring (FIG. 2C-E) (Chretien *et al., J Struct Biol.,* 120:117-133, 1997; Piel *et al., J Cell Biol.,* 149:317-330, 2000). Based on its centriolar localization the protein was named centriolin. Centriolin was also found at noncentrosomal apical bands of material in specialized epithelial cells that lack proteins involved in microtubule nucleation (g tubulin) and appear to anchor the minus ends of microtubules (Mogensen *et al., Cell Motil Cytoskel,* 36:276-290, 1997) (FIG. 2F and G).

Example 2. Centriolin Antibodies and Altered Centriolin Levels Induce Delays and Defects in Cytokinesis

As an initial test of centriolin function, the effects of affinity-purified anti-centriolin antibodies were examined following microinjection into developing *Xenopus* embryos. A centriolin antibody that cross-reacted with the *Xenopus* protein on Western blots and stained *Xenopus* tissue culture cells by immunofluorescence (FIG. 1A) was injected into one cell of two-cell embryos as described (Doxsey *et al., Cell,* 76:639-650, 1994). Uninjected and preimmune IgG-injected cells divided normally, while cells injected with centriolin antibodies failed to cleave or cleaved a few times then arrested (FIG. 3A and B). Centriolin antibody-injected cells arrested with two nuclei and two microtubule asters indicating that they had accomplished chromosome segregation, nuclear reformation and centrosome duplication, but failed to complete cytokinesis.

Centriolin function was next tested in vertebrate cells by ectopically expressing protein from a full-length centriolin cDNA. The population of cells expressing hemagglutinin-tagged (HA) centriolin had a significantly higher percentage of telophase cells compared to cells expressing HA alone (FIG. 3E). A striking feature of these cells was the presence of microtubule bundles located within intercellular bridges despite the fact that the nuclei had reformed and contained decondensed chromatin (FIG. 3C and F). In control cells, nuclei with similar features were seen only in late-stage telophase cells with narrow intercellular bridges and midbodies, or in G1 cells (FIG. 3D and F). This suggested that cells expressing centriolin had exited mitosis but had not yet cleaved. One consequence of these delays and defects in cytokinesis was formation of binucleate cells apparently by coalescence of two nascent daughter cells into one (FIG. 3G).

To test the requirement of centriolin protein in cytokinesis we reduced its levels using small interfering RNAs (siRNAs) (Elbashir *et al., Nature,* 411:494-498, 2001). Treatment of telomerase-immortalized diploid human retinal pigment epithelial cells (RPE1) (Morales *et al., Nat Genet,* 21:115-118, 1999) with centriolin-specific siRNAs caused a ~5-fold reduction in centriolin mRNA levels (FIG. 4A). Although we were unable to examine protein levels by Western blotting of whole cell lysates due to the rare nature of this and other centrosome autoantigens (Doxsey *et al., Cell,* 76:639-650, 1994), immunofluorescence staining demonstrated that centriolin was undetectable or greatly reduced at centrosomes in most cells (FIG. 4B). Quantitative analysis showed that immunofluorescence signals at individual centrosomes was significantly below those in cells treated with control lamin A/C siRNA despite severe disruption of the nuclear lamina in the latter (FIG. 4C and D, Elbashir *et al., Nature,* 411:494-498, 2001).

Twenty four hours after treating RPE1 cells with siRNAs targeting centriolin an increase was observed in the percentage of cells in cytokinesis, the appearance of cells undergoing abnormal cytokinesis (wide intercellular bridges, persistent intercellular connections into G1), and formation of binucleate cells (FIG. 4E and F). Progression through mitosis was also examined by synchronizing a subpopulation of cells. Cells were first treated with siRNAs then with nocodazole to induce mitotic arrest. Upon removal of the drug, cells treated with lamin siRNAs transited through mitosis and completed cytokinesis within 60-80 minutes (similar to untreated cells). In contrast, cells treated with siRNAs targeting centriolin transited normally through prometaphase, metaphase, and early anaphase but were delayed in telophase by up to 20-40 minutes (FIG. 4G, panel 2). Similar results were obtained when nocodazole-treated mitotic cells were mechanically isolated from culture dishes and re-plated. The isolated cells remained in cytokinesis up to 45 minutes longer than controls (FIG. 4G, panel 3).

Cytokinesis defects and delays were also observed in another human cell line (HeLa), with a second set of siRNAs targeting a different centriolin sequence and with morpholino antisense DNA oligonucleotides targeting centriolin in a third cell line (HME1).

Example 3. Centriolin Shares Homology to Yeast Proteins of the MEN and SIN

Insights into the mechanism by which centriolin induced cytokinesis delays and defects came from analysis of the centriolin cDNA sequence (see experimental procedures). The full-length centriolin cDNA contained 6975 nucleotides with an open reading frame of 2325 amino

acids and predicted a molecular weight of 269 kD, consistent with the molecular weight of endogenous centriolin (FIG. 1A).

The amino acid sequence of centriolin had several interesting features (FIG. 5A). It predicted a protein with several coiled-coil regions interrupted by noncoiled domains (Lupas, *Science,* 252:1163-1164, 1991). Two domains within the centriolin sequence shared homology with human oncogenic transforming acidic coiled coil proteins (TACC) that localize to centrosomes and are implicated in microtubule binding and stabilization and spindle function (Gergely *et al., Proc Natl Acad Sci U S A,* 97:14352-14357, 2000). Centriolin amino acids 435-623 and 1385-1658 were 24% identical/47% similar and 20% identical/41% similar to the C-terminal half of TACCs, respectively. In addition, centriolin amino acids 879-913 were 40% identical/51% similar to amino acids 72-106 of human stathmin, an oncogenic protein involved in microtubule destabilization (Lee *et al., Nat Cell Biol.,* 3:643-649, 2001). The carboxyl terminus of centriolin was identical to CEP110, a naturally occurring fusion to the fibroblast growth factor (FGF) receptor that localizes to centrosomes, is oncogenic and is of unknown function (Guasch *et al., Blood,* 95:1788-1796, 2000). CEP110 may be a smaller isoform or truncated form of centriolin.

A domain of centriolin at the amino terminus (amino acids 126-234) shared homology with the carboxyl termini of budding yeast Nud1p and fission yeast Cdc11p (FIG. 5B and C). The centriolin sequence shared the same degree of homology to each of the yeast sequences (31-35% identical, 47-50% similar) as the yeast sequences did to one another (31% identical, 48% similar). Nud1p and Cdc11p are spindle pole body/centrosome proteins that anchor components of the yeast MEN and SIN, respectively, and they are required for completion of mitosis and cytokinesis (Bardin and Amon, *Nat Rev Mol Cell Biol.,* 2:815-826, 2001; Guertin *et al., Microbiol Mol Biol Rev,* 66:155-178, 2002; McCollum and Gould, *Trends Cell Biol.,* 11:89-95, 2001; Pereira and Schiebel, *Curr Opin Cell Biol.,* 13:762-769, 2001). A regulatory pathway homologous to the yeast MEN and SIN has not been identified in vertebrate cells.

Example 4. The Nud1 Domain of Centriolin Interacts with the Yeast Bub2p

The shared sequence homology between centriolin and Nud1p/Cdc11p and the related roles of the human and yeast genes in the completion of cytokinesis suggested that centriolin functioned in a vertebrate pathway analogous to the yeast MEN/SIN. Moreover, the centriolin

Nud1 domain contained the region of Nud1p that interacted directly with the downstream MEN/SIN component Bub2p.

To further examine the possibility that the centriolin Nud1 domain was a member of a pathway analogous to the MEN/SIN, its ability to interact with the yeast Bub2p was tested because no vertebrate Bub2p/Cdc11p has been unequivocally identified. Directed two-hybrid analysis revealed a specific interaction between the centriolin Nud1 domain and Bub2p (FIG. 5D). No signal was observed when either protein was used alone and no binding was detected between centriolin Nud1 and the budding yeast MEN component Bfa1p, consistent with observations in budding yeast (Pereira and Schiebel, *Curr Opin Cell Biol.*, 13:763-769, 2001). The centriolin Nud1-Bub2p interaction was confirmed by immunoprecipitation from yeast cells co-expressing the two proteins (FIG. 5E).

Example 5. Overexpression of the Centriolin Nud1-interacting Domain Induces Cytokinesis Defects

The results disclosed as part of this invention strongly suggested a role for centriolin and the Nud1 domain in particular, in regulating completion of cytokinesis. To test this more directly, the question of whether the 120 amino acid Bub2p-interacting Nud1 domain was sufficient to induce the cytokinesis defects observed in cells overexpressing the full-length protein was considered. The phenotype of cells overexpressing a GFP-tagged Nud1 domain was similar to that observed in cells expressing the full-length protein. There was a significant increase in the percent of telophase cells suggesting a delay in cytokinesis (FIG. 6D), and a dramatic increase in the proportion of binucleate cells suggesting a failure to complete cytokinesis (FIG. 6C and E). Importantly, overexpression of the Nud1 domain had no detectable effect on the centrosomal localization of the endogenous protein (FIG.. 6A and B). The most likely explanation of these results is that the cytoplasmic overexpressed Nud1 domain sequestered proteins related to the MEN/SIN preventing them from associating with centriolin at the centrosome and inducing delays and defects in cytokinesis.

Example 6. Reduction in Pericentrin-B Levels Mislocalizes Centriolin and Induces Cytokinesis Defects

In a search of other candidate genes in the putative vertebrate MEN/SIN, we identified pericentrin-B (Accession#AF515282) as a centrosome anchoring protein for centriolin. Pericentrin-B appears to be a larger isoform of pericentrin-A (Doxsey *et al.*, *Cell*, 76:639-650, 1994). It has a different molecular weight, cellular distribution and biochemical properties, although its function is unknown. Pericentrin-B co-localized with centriolin to the centrosome and, like centriolin, translocated to the intercellular bridge during cytokinesis (FIG. 7A). Cells treated with pericentrin-B-specific siRNAs showed a 5-fold decrease in mRNA levels and a significant loss of centrosome-associated protein (FIG. 7B-D). Essentially all centrosomes with reduced pericentrin-B also showed a dramatic reduction in centriolin (FIG. 7E; FIG. 11). Loss of centrosomal centriolin appeared to be selective since other centrosome proteins such as g tubulin were not significantly affected (100% positive; FIG. 11).

Cells treated with siRNAs targeting pericentrin-B exhibited cytokinesis delays and defects indistinguishable from those observed in cells with reduced centriolin levels (FIG. 7F and G). Moreover, mislocalization of pericentrin-B and consequently centriolin, by overexpressing the pericentrin-B centrosomal anchoring domain (Gillingham and Munro, *EMBO Rep*, 1:524-529, 2000) caused cytokinesis defects in COS-7 cells. Thus, the pericentrin-B-induced mislocalization of centriolin from centrosomes suggests that centrosomal anchoring of centriolin, rather than reduction in centriolin levels, was sufficient to induce cytokinesis defects just as mislocalization of spindle pole body-associated Cdc11p and Nud1p induce defects in mitotic exit and cytokinesis. Cytokinesis defects were also observed with a second set of pericentrin-B-specific siRNAs, and in several cell lines including HeLa (FIG. 7A-G), RPE1, and HME1.

Example 7. Reduction in Centriolin and Pericentrin-B Levels Induces G1/G0 Arrest

The cytokinesis defects and delays induced by siRNAs targeting centriolin and pericentrin-B in RPE1 cells were observed at early times after treatment (FIG. 4A-G, less than 24 hours). At later times (48-72 hours post treatment) a reduction in the mitotic index was observed suggesting that the cells were arrested at some other stage of the cell cycle. This was directly tested by treating cells with nocodazole to induce mitotic arrest. Although 71% of lamin siRNA-treated control cells arrested in mitosis, only ~1% of the centriolin and pericentrin-B siRNA-

treated cells arrested at this cell cycle stage. To determine the cell cycle stage of arrest, cells were analyzed by flow cytometry in the presence and absence of nocodazole. In the absence of nocodazole, cells treated with siRNAs targeting centriolin and pericentrin-B had a slightly higher percentage of cells in G1 compared with control cells treated with lamin or GFP siRNA (FIG. 9; see also FIG. 10). In the presence of nocodazole, control cells showed a significant shift from the G1 peak to the G2/M peak (FIG. 8A and B; FIG. 9). In contrast, cells treated with siRNAs targeting both centriolin or pericentrin-B did not significantly shift into the G2/M peak in the presence of nocodazole but remained largely in G1. The inability to undergo a nocodazole-induced shift into the G2/M peak was a feature shared by cells driven into G0 by serum starvation (FIG. 8C). These results demonstrate that cells treated with centriolin and pericentrin-B siRNAs arrest prior to S phase, possibly in G1/S, G1, or G0.

Another approach was used to examine the stage of cell cycle arrest. Ki-67 is an antibody directed against a nuclear protein that stains only cycling cells or cells arrested in cycle, including those arrested in G1/S or S phase (Gerdes *et al., J Immunol,* 133:1710-1715, 1984). Cells that exit the cell cycle and become quiescent (G0) or undergo differentiation, are Ki-67-negative. As expected, nearly all untreated cells or control cells treated with siRNAs targeting GFP or lamins A/C were cycling and thus, positive for Ki-67 (FIG. 8E). However, treatment with siRNAs targeting centriolin and pericentrin-B eliminated Ki-67 staining in essentially all affected cells (80-85%, FIG. 8D, arrowhead, E). Nearly identical results were observed in cells driven into G0 by serum starvation (95% negative). Taken together, the results from mitotic index assays, flow cytometry and Ki-76 staining demonstrated that reduction of centriolin and pericentrin-B levels by siRNA prevented cells from entering S phase and appeared to drive them out of cycle into a G0-like state. Cell cycle arrest was also observed in another diploid cell line (HME1).

Example 8. p53 Dependence of the G1/G0 Arrest

The G1/G0 arrest observed in cells with reduced centriolin and pericentrin-B seemed to be p53-dependent. Reduction of centriolin or pericentrin-B in cells with wild type p53 (RPE1, HME1) (Morales *et al., Nat Genet,* 21:115-118, 1999) caused G1/G0 arrest as shown by the lack of Ki-67 staining and inability of cells to shift from G1 to G2/M in the presence of nocodazole (FIG. 8A-E). In contrast, cells with abrogated p53 function (HeLa, Saos-2) (Scheffner *et al.,*

Cell, 63:1129-11, 1990; Shew *et al., Proc Natl Acad Sci U S A*, 87:6-10, 1990) did not arrest when centriolin or pericentrin-B levels were reduced. The majority stained for Ki-67 (FIG. 8F and G) and shifted into the G2 peak when treated with nocodazole.

Example 9. Rb Phosphorylation

The effect of the centriolin and pericentrin-B siRNA treatment on Ki-67 staining was not cell-type specific because similar results were observed in three diploid cell lines (*i.e.*, RPE, BJ-1, MRC-5), and two genetically unstable tumor-like cell lines with altered p53 (*i.e.*, HeLa, COS). Similar results were obtained in cells overexpressing centriolin but not the Nud1 domain alone.

Example 10. Centriolin Silencing by siRNA Induces Cytokinesis Failure and a Novel Cytokinesis Phenotype

To determine the function of centriolin, its levels were reduced using siRNAs (Fire *et al.*, *Nature*, 391:806-811, 1998; Elbashir *et al.*, *Nature*, 411:494-498, 2001). Treatment of telomerase-immortalized diploid human retinal pigment epithedial (RPE-1) cells (Morales *et al.*, *Nature Genetics*, 21:115-118, 1999) with centriolin-specific siRNAs caused a significant reduction in centriolin mRNA levels (FIG. 4A). Although protein levels were not successfully examined by Western blotting of whole cell lysates due to the rare nature of this and other centrosome autoantigens (Doxsey *et al.*, *Cell*, 76:639-650, 1994), immunofluorescence staining demonstrated that centriolin was undetectable, or greatly reduces, at centrosomes in most cells (86%; n = 1,012). Quantitative analysis showed that immunofluorescence signals at individual centrosomes were significantly below those in cells treated with control lamin A/C siRNA, despite severe disruption of the nuclear lamina in the latter (FIG. 4B and D) (Elbashir *et al.*, *Nature*, 411:494-498, 2001). Midbody staining of centriolin was also reduced in cells treated with siRNAs targeting centriolin.

Because centriolin shares homology with proteins known to affect microtubule organization and cytokinesis, we examined cells with reduced centriolin for defects in these functions. The most obvious cellular change detected in RPE-1 cells with reduced centriolin was a dramatic increase in the percentage of late-state mitotic cells (~70-fold increase; FIG. 4E). In addition, an increase in the percentage of binucleate cells was observed in three different cell lines, suggesting that a certain proportion of cells failed to cleave (FIG. 15B). The incidence of

binucleate cells was significantly greater than controls, although somewhat lower than that observed for some other proteins involved in cytokinesis (Matuliene and Kuriyama, *Mol. Biol. Cell.*, 13:1832-1845, 2002; Meraldi *et al.*, EMBO. J., 21:483-492, 2002; Mollinari *et al.*, *J. Cell. Biol.*, 157:1175-1186, 2002). A similar cytokinesis phenotype was observed with a second set of siRNAs targeting a different centriolin sequence and with morpholino antisense DNA oligonucleotides targeting centriolin.

The dramatically high percentage of cells in late mitotic stages suggested a unique cytokinesis defect in these cells. When carefully analyzed by immunofluorescence microscopy, cells with reduced centriolin appeared to be arrested or delayed in the final stages of cytokinesis. Most cells retained intercellular bridges of varying length and thickness (FIG. 15M and N, arrowheads). In some cases, cells remained connected even though one or both of the future daughter cells had reentered mitosis (FIG. 15M and N). Some cells failed to cleave, forming syncytia with two, three, of four cells remaining interconnected (FIG. 15M and N). During the early stages of cytokinesis, midbodies appeared normal.

A more complete understanding of the mechanism of cytokinesis failure was obtained by imaging live HeLa cells treated with centriolin-specific siRNAs (FIG. 16A-F; see Videos 1-3, available at http://www.jcb.org/cgi/content/full/jcb.200201105/DC1). As expected, control cells (lamin siRNA) performed a distinct cell cleavage event with normal timing (average 2 h after mitosis) and immediately flattened and crawled apart (FIG. 16A). Cells silenced for centriolin progressed normally through mitosis (FIG. 15G-J; FIG. 16E) and sometimes cleaved normally, but most failed to cleave or cleaved after prolonged periods of time (up to 23.2 h after metaphase; FIG. 16B-D and F). These cells arrested or delayed in a unique post-telophase state. Most were unusually elongated, each with a persistent intercellular bridge of variable diameter that was often dynamic. Bridges alternated between thin threads of interconnecting cytoplasm to very thick interconnections of large diameter that appeared able to produce membrane ruffles (FIG. 16B, 5:50, arrow). Midbodies were not detected within persistent interconnections between cells, suggesting that they were lost sometime during the protracted period spent in cytokinesis. Interconnected cells sometimes coalesced to form single cells and then quickly moved apart again (FIG. 16D). They sometimes made multiple failed attempts at cleavage, but in no case did we observe a cell that formed a stable binucleate. This suggested that binucleate cells observed in fixed cells (FIG. 15B-D) were transient intermediates in a process that involved

multiple failed attempts at cytokinesis. Cells that retained intercellular connections for long periods of time continued to progress through the cell cycle. To our surprise, some cells reentered the next mitosis while still interconnected and produced interconnected "progeny" that formed two- to four-cell syncytia, thus confirming the cell-cell interconnections observed by indirect immunofluorescence (FIG. 15M and N). In some cases, cells that remained interconnected for long periods of time appeared to undergo apoptosis. They showed extensive blebbing, increased phase density, and decreased size and lifted from the substrate (FIG. 16B, upper cell, 7:20).

Microtubule organization in cells with reduced centriolin appeared normal at all cell cycle stages. This included microtubules of the spindle midzone in anaphase and the Midbody in telophase (FIG. 15E-J). Microtubule nucleation from centrosomes also appeared normal (FIG. 15K and L), although a slight delay was sometimes observed within the first minute or two. γ-Tubulin, a marker for centrosome-associated microtubule nucleation, was localized normally to centrosomes (FIG. 4B and D), as were several other centrosome antigens, including GCP-2 (Murphy *et al.*, *J. Cell Biol.*, 141:663-674, 1998) and cNap-1 (Fry *et al.*, *J. Cell Biol.*, 141:1563-1574, 1998; unpublished data). Midbody markers, such as anillin (see Glotzer, *Annu. Rev. Cell. Dev. Biol.*, 17:351-386 2001) and γ-tubulin (Shu *et al.*, *J.Cell. Sci.*, 108:2955-2962, 1995), were also localized normally. At later stages of cytokinesis in cells with long intercellular bridges, midbodies were no longer detected. These data indicate that cytokinesis failure did not result from disruption of microtubules, centrosomes, or midbodies.

Example 11. The Centriolin Nud1 Domain Interacts with the Yeast Bub2p *In Vitro*

Budding yeast Nud1 p anchors the MEN to the spindle pole body through direct interactions with Bub2p and perhaps other MEN components (Gruneberg *et al.*, *EMBO J.*, 19:6475-6488, 2000; Pereira *et al.*, *Mol. Cell.*, 6:1-10, 2000). To determine if the centriolin Nud1 homology domain (FIG. 5B) had similar properties, its ability to bind Bub2p by directed two-hybrid analysis and immunoprecipitation was tested. Because no vertebrate Bub2p homologue has been unequivocally identified (Cuif *et al.*, *EMBO J.*, 18:1772-1782, 1999), the ability of the centriolin Nud1 domain to interact with yeast Bub2p was examined. Both two-hybrid analysis and immuno-precipitation from yeast cells coexpressing the two proteins revealed a strong and specific interaction between the centriolin Nud1 domain and Bub2p

(FIG. 5D and E). No signal was observed when either protein was used alone, and no binding was detected between the centriolin Nud1 domain and the budding yeast MEN component Bfa1p, consistent with interaction observations in budding yeast (Pereira and Schiebel, *Curr. Opin. Cell Biol.*, 13:762-769, 2001).

Example 12. Cleavage Failure is Observed in *Xenopus* Embryos Injected with Centriolin Antibodies

Another approach was used to examine centriolin function. When affinity-purified anticentriolin antibodies (FIG. 1A) were microinjected into one cell of two-cell *Xenopus* embryos (Doxsey *et al.*, *Cell*, 76:639-650, 1994), the injected cell failed to cleave, or cleaved a few times and then arrested; uninjected cells or preimmune IgG-injected cells divided normally (FIG. 3A and B). Centriolin antibody-injected cells arrested with two nuclei and two well-organized microtubule asters, indicating that karyokinesis and microtubule organization were normal, but cells failed to complete the final event of mitosis, cell cleavage (FIG. 17B). Preimmune IgG-injected cells had a single nucleus with one or two microtubule asters, depending on their cell cycle stage, as would be expected for cells that had undergone normal cell cleavage (FIG. 17A). Taken together, the results from gene silencing, antibody injection, and protein overexpression in several experimental systems all demonstrate that centriolin plays an important role in the late stages of cytokinesis.

Example 13. SiRNA-Induced Gene Silencing of Centriolin Causes G1/G0 Arrest

Cytokinesis defects and delays induced by centriolin silencing were observed at early times after treatment of RPE-1 cells (18-24 h). At later times (48-72 h after treatment), a reduction in the mitotic index was observed, suggesting that the cells were arrested at some other stage of the cell cycle. This was directly tested by treating cells with nocodazole to induce mitotic arrest and quantifying mitotic cells in DAPI-stained preparations. Under these conditions, most lamin siRNA-treated control RPE-1 cells were arrested in mitosis (71%), whereas only a small fraction of centriolin siRNA-treated cells arrested at this cell cycle stage (<1%). To determine the cell cycle stage of arrest, cells were analyzed by flow cytometry. In the presence of nocodazole, control cells showed a significant shift from the G1 peak to the G2/M peak (FIG. 8A, red). In contrast, cells treated with siRNAs targeting centriolin did not

significantly shift into the G2/M peak in the presence of nocodazole but remained largely in G1 (FIG. 8A, blue). The inability to undergo a nocodazole-induced shift into the G2/M peak was a feature shared by cells driven into G0 by serum starvation (FIG. 8C, blue). The proportion of cells in S phase was either unaltered or slightly decreased in cells silenced for centriolin both in the presence of nocodazole (centriolin, 13%; lamin, 23%) or in its absence (centriolin, 13%; lamin, 19%). These results demonstrate that cells with reduced centriolin arrest before S phase, possibly in G1/S, G1, or G0.

Ki-67 staining was also used to examine the stage of cell cycle arrest. Ki-67 is an antibody directed against a nuclear protein that stains cycling cells or cells arrested in cycle (*e.g.*, C1/S or S phase; Gerdes *et al.*, *J. Immunol.*, 133:1710-1715, 1984) but not cells that are quiescent (G0) or differentiated. As expected, nearly all untreated RPE-1 cells or control cells treated with siRNAs targeting GFP or lamins A/C were positive for Ki-67 (FIG. 8D and E). However, most cells with reduced centriolin had undetectable levels of Ki-67 staining (FIG. 8D and E). Taken together, results from mitotic index assays, flow cytometry, and Ki-76 staining in RPE-1 and HME-1 (human mammary epithelia) cells (unpublished data) demonstrated that reduction of centriolin levels prevented cells from entering S phase and appeared to drive them out of cycle into a G0-like state. This cell cycle arrest effectively prevents the initiation of additional rounds of centrosome duplication in cells compromised by having diminished levels of centriolin.

OTHER EMBODIMENTS

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.